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Ecological and quantitative studies of occurrence of soybean Sclerotinia stem rot, caused by Sclerotinia sclerotiorum, in the North-Central Region of the United States

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Ecological and quantitative studies of occurrence of soybean Sclerotinia stem rot, caused by *Sclerotinia sclerotiorum*, in the North-Central Region of the United States

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ABSTRACT

Since the early 90's, Sclerotinia stem rot of soybeans (SSR), caused by *Sclerotinia sclerotiorum* (Lib.) de Bary, has emerged as a serious disease problem in the north-central soybean production region of the United States. Contributing factors are recent changes in cultural practices in the region, such as increased use of reduced tillage, narrow soybean rows and early planting. The increased SSR occurrence in the North-Central Region of the United States, the major US soybean production region, is of concern because of both the scarcity of resistant cultivars and the cost of fungicides. It has been reported that each 10% increase in SSR incidence may reduce yield by 147 to 263 kg/ha (in Illinois), by 235 kg/ha (in Michigan) or by 170 to 330 kg/ha (in Iowa). Understanding the epidemiology of *Sclerotinia sclerotiorum* associated with management practices and developing models that explain, and eventually may forecast, the risk of SSR occurrence in the region may help extension specialists and growers manage the disease.

Regional prevalence of soybean Sclerotinia stem rot (SSR) was modeled using historical data collected between 1995 and 1998 from 4 states of the North-Central Region of the United States (Illinois, Iowa, Minnesota and Ohio). Tillage practices, soil texture, and summer weather variables from the National Oceanic and Atmospheric Administration (average monthly air temperature and total precipitation during July and August) were used as input variables. Prevalence was defined as the percentage of fields in which SSR was found. Differences between states in disease prevalence were addressed, using regional indicator variables. Logistic regression was used to estimate the probability of stem rot prevalence in the four states. A developed model had high explanatory power (77.8%). To

validate our model, each state was divided into small geographic areas, and disease prevalence was estimated for each area. The R^2 of the regression analysis between observed and estimated SSR prevalence was 0.71.

Incidence, which was defined as percentage of infected soybean plants in a field, was also modeled. When the input variables for prevalence were tested for their ability to explain the within-field SSR incidence, only a small proportion of the SSR incidence was explained. The R^2 of the regression analysis between observed and estimated SSR incidence was 0.065, and predicted incidence was consistently higher than observed incidence. Our results suggest that use of selected variables for prediction of regional prevalence would be feasible but not for prediction of field incidence, and a different site-specific approach should therefore be followed.

We used Bayesian methodology to investigate the level of uncertainty associated with the parameter estimates derived with the logistic regression analysis of regional SSR prevalence. Bayesian analysis suggests that the four-year data set used in the analysis may not be informative enough to produce reliable estimates of the effect of some explanatory variables on SSR prevalence. The variable most sensitive to extra-sample incorporated information was precipitation of August. The logistic regression analysis demonstrated that the effect of August precipitation was not statistically significant for the prevalence of SSR in the North Central Region of the United States. However, during the four years of the survey, precipitation during July and August was always at least as high as the 30-year average for the region. Therefore, Bayesian analysis suggests that the data set used in the present analysis may not account for regional extreme weather pattern (i.e. precipitation of summer higher or lower than the 30-year average for the region).

In addition, the relationships between management practices, weather variables and soybean yield were examined using multiple linear regression to investigate whether high potential yield environments are also high SSR risk environments. Occurrence of SSR was indeed found to be strongly associated with high attainable yield. With these findings, farmers' decisions on SSR management were examined using decision theory under uncertainty. Bayesian decision procedures were used to combine information from our model (prediction) with farmers' subjective probability of SSR incidence (personal estimates, based on farmers' previous experience on SSR incidence and strength of risk aversion). *MAXIMIN* and *MAXIMAX* criteria were used to incorporate farmers' site-specific prior experience on SSR incidence. The *MAXIMIN* criterion corresponds to farmers with prior experience indicating that their fields should be the ones under high SSR risk. The *MAXIMAX* criterion corresponds to farmers with prior experience indicating that their fields should be the ones under low SSR risk. Optimum actions were derived using the criterion of profit maximization. Our results suggest that management practices would be selected so as to increase attainable yield, despite the association of the practices with high disease risk.

Differences in soil temperature and water potential have been observed under different combinations of tillage regimes and planting row widths, with soil surface temperature and moisture fluctuating more under the combination of no tillage and wide rows than under other combinations. Experiments were conducted to determine the effects of soil temperature and water potential fluctuations on sclerotium germination, apothecium production and fungi isolated from the surface of *S. sclerotiorum* sclerotia. Our results showed that: i) small temperature fluctuations increased sclerotium germination and

apothecium production compared to large or no temperature fluctuations; ii) moisture fluctuations were detrimental to sclerotium germination and apothecium production, with the constant saturation treatment yielding the highest number of germinated sclerotia and apothecia. Moreover, there were differences in the fungal species isolated from sclerotia surface with *Fusarium spp.* being the predominant species isolated in the temperature fluctuation experiments, and *Penicillium spp.* being the predominant species isolated in the moisture fluctuation experiments. Our results may provide clues to explain the effects of different tillage systems and widths of planting rows on apothecium production and levels of SSR incidence.

CHAPTER I. INTRODUCTION

A. Soybean production

The soybean [*Glycine max* (L.) Merrill] is one of the most economically and nutritionally valuable food commodities of the world, since it is the most cost-effective crop with respect to combined protein and calorie yields per unit of resource invested (20). Soybean supplies one-forth of the world's fats and oils, about two-thirds of the world's animal feed protein and three-fourths of the world trade in high protein meals. There is an upward trend in world soybean-planted acreage (10% increase during the last decade) and in average soybean yield per hectare (13.2% increase for the period 1989-1992 compared to 1978-1981). North and Central America combined takes first place in soybean production, with South America in second and Asia in third place (28).

The United States is one of the world's largest soybean exporters. In the 1999/2000 trade year, soybean and soybean product exports accounted for about 40% of the U.S. soybean production. During that year Brazil and Argentina were the second and third largest exporters of soybean, respectively, behind the United States. China, though the world's fourth-largest producer, was one of the major soybean importers because of the rapid growth of its economy, which has increased food consumption. However, in the 2000/2001 trade year, Brazil was the world's largest soybean exporter and the United States moved to second place (*Source*: USDA-NASS).

B. Sclerotinia stem rot

Diseases are a major cause of soybean losses world wide (31,32). In recent years, Sclerotinia stem rot of soybean (SSR), caused by *Sclerotinia sclerotiorum* (Lib.) de Bary, has emerged as a leading cause of soybean yield losses in the north-central soybean production region of the United States. Before 1990's, the disease was known to cause only localized epidemics in Michigan, Minnesota, and Wisconsin (14). In the same region, SSR was ranked 12th as a cause of yield losses in 1990 (8) and second in 1994 (31). In a survey conducted between 1995 and 1998 in five states of the North Central Region of the United States (Illinois, Iowa, Minnesota, Missouri and Ohio), SSR was prevalent in 1996 (30).

The importance of Sclerotinia stem rot to soybean yield losses has generated several research efforts in the North-Central Region of the United States. These efforts include understanding the life cycle of the pathogen, identifying resistant or tolerant varieties, and investigating the influence of production practices such as row spacing, tillage and chemical applications on SSR development and management.

C. Research rationale

Before 1990, in the North-Central soybean production region of the United States, Sclerotinia stem rot was causing only localized epidemics in areas of Michigan, Minnesota and Wisconsin (10,12,13) where soybeans of maturity groups 0 to I are grown. Since 1990, outbreaks of SSR have become more frequent and severe (8,30,32) and have expanded into areas where maturity groups II to III are grown. In Iowa the disease was not recognized as a significant problem until 1992, when severe outbreaks occurred in major soybean production areas of northern and central Iowa (33). In the 1996 growing season, the disease was in epidemic proportions in Iowa (30), much more severe than in 1992 and 1994 (33). It has been reported that every 10% increase of SSR incidence may reduce yield by 147 to 263 kg/ha in Illinois (15), by 235 kg/ha in Michigan (5) or by 170 to 330 kg/ha in Iowa (33).

Management practices--including narrow row spacing, increased plant populations, early planting dates and high soil fertility--that are intended to increase soybean yield, create favorable conditions for SSR development within the crop canopy (17). Shortened crop sequences, frequently limited to corn and soybean, reduced tillage, or rotation with susceptible crops such as sunflower, canola, or dry beans are factors that increase the soil inoculum density and may also contribute to the increased SSR occurrence in the North-Central Region of the United States (17,24). Finally, above-normal precipitation and lower temperatures occurring during the critical infection period following flowering also favor SSR development (30).

The increase in Sclerotinia stem rot in the North-Central Region of the United States, the major soybean production region, is of concern for two reasons: the scarcity of resistant cultivars in the maturity groups appropriate for the region and the cost of fungicides for control of Sclerotinia stem rot (4,11,13,19). Sclerotinia stem rot can be controlled successfully by fungicides in susceptible crops such as dry bean and canola (26) but chemical control of SSR in soybean has not proven economically feasible (4,10). As a result, strategies for controlling SSR in soybean emphasize cultivar selection and management practices that reduce canopy density. However, complete resistance to SSR has not been reported (4,10,12). Thus, it seems that implementation of management practices is critical to SSR control at present.

It has been suggested (2,3) that detailed and quantitative epidemiological data would be essential for developing effective and economical control programs for diseases caused by

Sclerotinia spp. Information on the role of moisture and other weather factors on the production of ascospores and infection of beans has been used to forecast white mold in New York and Nebraska (2). This disease-forecasting system could improve timing of, and application procedures for fungicide sprayings of lettuce and beans (16,18). For Sclerotinia stem rot of soybean in the North-Central Region, quantitative epidemiological data would help us (i) identify the factors associated with outbreaks of SSR, and (ii) evaluate whether these factors can be used to predict SSR outbreaks in this region.

Such a prediction system seems necessary in the case of SSR, in which disease management is based on cultural practices most of which are implemented long before SSR occurs in a field. Tillage is applied in late fall, about 9 months before the next soybean growing season, seed is purchased in December, about 3 to 4 months before planting, and decisions on row planting are made in late spring. An additional difficulty in SSR management is the fact that cultural practices not only affect disease occurrence but also are related to attainable soybean yields. There is a general notion in the North-Central Region of the United States that SSR occurrence is highly related to high-yield environments (12,17).

An attempt in Wisconsin (27) to predict white mold incidence of snapbeans was made using cultural, environmental parameters and field history as inputs. The models involved selection of fields with low white mold potential and determination of the necessity of fungicide applications during two critical periods: 7 days prior to and 7 days after 10% bloom. Three years of data were analyzed, using the cropping history, irrigation frequency, row width, evapotranspiration, heat units, canopy density, rainfall/irrigation and stand density as the independent variables. The models (one for before and one for after bloom) had low explanatory power (R^2 equal to 27.4 and 35, respectively).

Besides the need to develop an SSR-forecasting system, there is a need to understand the epidemiology of this pathosystem so that the role of cultural practices on SSR development could be thoroughly explained. Deep plowing has been recommended for control of white mold –at least during the year following plowing (29). No-tillage reduces significantly SSR occurrence (17,29,30). Fewer apothecia have been found with no-tillage (9,17). Kurle et al. (17) concluded that canopy density differences are more important than differences in numbers of viable sclerotia among tillage systems. Effects of row spacing, growth habit, and plant density on canopy development, disease incidence and severity were also reported in bean fields in Nebraska (7,25) and in potato fields in New York (21). Planting in wide rather than in narrow rows is an effective management practice for SSR (25).

Cook et al. (6) noted that the majority of sclerotia that form initials in soil were at a depth of 5 to 10 cm. Similar results were reported by Radulescu and Crisan (23). However, mature apothecia with stipes longer than 3 cm are rarely produced under field conditions, and this characteristic may act as a physical limitation of the soil depth to which apothecia can be a source of inoculum (22). It seems that two factors, aeration and moisture are probably involved with burial depth. (22). Extreme drying and possibly high temperature have prolonged detrimental effects on apothecial production, although the sclerotia remain viable (1). It was suggested that in no-tillage, sclerotia remain on the soil surface and thus are exposed to extensive drying conditions that may affect germination, whereas in minimum tillage, sclerotia are placed close to the soil surface where soil does not dry out as extensively as on the soil surface (30).

Soil moisture and temperature, especially within the top 2-3 cm of soil, vary considerably and are affected by parameters such as wind velocity, type and density of plant canopy or management practices such as tillage and row spacing. Workneh and Yang observed that soil temperature and moisture fluctuated more on the soil surface than at small depths (0 to 5 cm) and more with wide (30 cm) than with narrow (15 cm) rows (*unpublished data*). They observed that the coolest and wettest soil is with narrow row planting at 5 cm depth, and the warmest and driest is at the soil surface with wide row planting.

D. Research objectives

The objectives of this research were:

1. to investigate the effect of fluctuations of soil temperature and soil water potential, on sclerotia germination and apothecia production of *S. sclerotiorum*,
2. to develop explanatory models of SSR prevalence in four states of the north-central region and to account for uncertainty associated with these models, and
3. to investigate the relationship between yield and production variables that affect SSR occurrence and to examine soybean farmers' production decisions in relation to SSR incidence, using decision theory under uncertainty.

E. Dissertation organization

This dissertation consists of an abstract and six chapters. Chapter 1 is a general introduction to the importance of soybean as a worldwide feeding crop, rationale of the research, and research objectives. Chapter 2 is a literature review on the biology, ecology, epidemiology and control of *Sclerotinia sclerotiorum*. Chapter 3 reports research on the effects of fluctuations in soil temperature and soil water potential on sclerotia germination, apothecium production and frequency of sclerotia parasitic fungi of *S. sclerotiorum*. Chapter

4 presents a study on modeling the prevalence of soybean Sclerotinia Stem Rot in the North Central Region of the United States, using data collected from a survey in 5 states (Illinois, Iowa, Minnesota, Missouri and Ohio) between 1995 and 1998. Chapter 5 presents a study on uncertainty related to parameter estimates derived from the modeling described in Chapter 4, using Bayesian methodology. Chapter 6 presents a study on the effect of management practices on prevalence of soybean Sclerotinia stem rot and soybean yield in the North-Central Region of the United States and possible farmers' decisions under uncertainty. Chapter 7 summarizes research results and suggests subject areas for further investigation.

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CHAPTER II. LITERATURE REVIEW

A. Biology, ecology, and epidemiology of *Sclerotinia spp.*

History and nomenclature. The history of *Sclerotinia sclerotiorum* (Lib.) de Bary has been described in detail by Purdy (80). Madame M.A. Libert first described the pathogen and named it *Peziza sclerotiorum*, in 1937. Later, Fuckel erected and described the genus *Sclerotinia* (80). He honored Madame Libert by renaming *Peziza sclerotiorum* as *Sclerotinia libertiana*. This binomial was accepted and was used until 1924, when Wakefield (97) showed that it was inconsistent with the International Rules of Botanical Nomenclature and cited G.E. Massee as the proper authority for the name *Sclerotinia sclerotiorum* (Lib.). Massee had used the binomial in 1895, but de Bary had used it in his 1884 contribution (31). Thus, the proper name and authority for the pathogen is *Sclerotinia sclerotiorum* (Lib.) de Bary. In 1972, Korf and Dumont (55), on the basis of anatomical studies, proposed the transfer of the epithet to the genus *Whetzelinia*. Thus, the binomial *Sclerotinia sclerotiorum* (Lib.) de Bary is equivalent to *Whetzelinia sclerotiorum* (Lib.) Korf & Dumont. The generic and species characters important in the taxonomy of *S. sclerotiorum* have been discussed by Kohn (56). Currently, *S. sclerotiorum* is classified as follows: Kingdom Fungi; Phylum Ascomycota; Class Ascomycetes; Order Helotiales; Family Sclerotiniaceae; Genus *Sclerotinia*; Species *sclerotiorum* (7).

Sclerotinia stem rot (SSR) of soybeans, first reported in Hungary in 1924, was first reported in the United States in 1946 (24,101). It has since been reported in Argentina, Brazil, Canada, China, India, South Africa and other soybean-growing areas (40). In the North-Central Region of the United States, the disease was a minor problem prior to 1990, with localized epidemics occurring in areas of Michigan, Minnesota and Wisconsin where soybeans of maturity groups 0 and I are grown (39). Since the beginning of the last decade, the prevalence and intensity of the disease in the North-Central United States has drawn attention from growers, researchers, and extension personnel (41). The disease has been causing problems farther south, where soybean of maturity groups II and III are grown (109).

Host range and geographic distribution. *Sclerotinia sclerotiorum* appears to be among the most non-specific, omnivorous, and successful plant pathogens. Boland and Hall (17) compiled an index of *S. sclerotiorum* hosts that includes 75 families, 278 genera, 408 species, and 42 subspecies or varieties. Most hosts of *S. sclerotiorum* are herbaceous, dicotyledonous angiosperms, but several are monocots (17,79). *S. sclerotiorum* occurs in relatively cool and moist areas, but it also occurs in areas that are generally hot and dry (80). The disease has been reported from many countries located in all continents. It is probable that the fungus occurs in almost every country (80).

Sclerotium formation. De Bary (31) described in detail the process of sclerotia development when the fungus is cultivated on a microscopic slide. Sclerotium formation and composition was discussed later by Le Tourneau (60) as well. When growth of a colony of *S. sclerotiorum* is restricted, such as when it reaches the edge of a container, the mycelial mat thickens and produces white mounds of mycelium covered with small liquid droplets. Sclerotium increases in size by formation of new hyphal cells and expansion of existing cells. Then hyphal cell membranes thicken, and the tissue differentiates into an inner medulla of loosely packed hyphae and an outer cortex of thin-walled compact cells (21,27). The differentiation process is centrifugal, commencing in the interior of the tissue and advancing rapidly towards the circumference. Maturation is marked by surface delimination and formation of liquid droplets (27). The sclerotium is delimited by formation of an external rind (21).Within one week or so, the process is completed and a mature sclerotium can be removed from the substrate (60). Sclerotia may also be formed in concentric circles or other regular patterns over the substrate as the result of endogenous rhythms (51).

Generally, the greatest portion of the dry matter of mature sclerotia is made up of carbohydrates, mainly β-glucan, chitin, glycogen, glucose, fructose, mannitol, and trehalose (60). Other constituents are crude fat (2%), ash (3.5-5%), and protein (20-25%). Nutritional factors such as carbon and nitrogen availability may affect sclerotium formation and growth (98,99). There are composition differences between sclerotia grown in culture and those obtained from field collections (60). Non-nutritional factors, such as light, temperature, pH, atmospheric composition, and osmotic potential also affect sclerotium formation (104). Light has been found to be associated with higher numbers of formed sclerotia (90), but dry weight per sclerotium was greater when *S. sclerotiorum* was cultured in darkness (51). *S. sclerotiorum* grows and produces sclerotia over a range of temperatures from near 0 to 30 ^{0}C and over a pH range of 2.5-9 (69). Mycelial growth can be stimulated by osmotic potentials of -1 to -14 bars, although it slowly decreases below this level, and sclerotia form at -65 bars but not at -73 bars (42).

Some isolates of *S. sclerotiorum* may lose their ability to form sclerotia after repeated subculturing, due to fungus inability to synthesize specific compounds (60). It has been suggested that sclerin, a known metabolite of *Sclerotinia spp.*, may be involved in formation of melanin and hyphal aggregates (60). Also, inhibitors may play a role in preventing sclerotium formation (60).

Sclerotium survival. *S. sclerotiorum* survives in the soil and in plant debris as sclerotia. Sclerotia contain reserve food materials and allow *S. sclerotiorum* to survive for long periods of time under adverse conditions (5). Halkilahti (43) found that survival of sclerotia on the soil surface depends on weather conditions in summer: in dry summers they survive well, but in wet summers high temperatures favor their destruction. Furthermore, it was reported that sclerotia survive better in cropped than in noncropped soils (81,105). Adams (4) found that cropping sequence had no effect on inoculum density and he observed good survival of sclerotia for more than 15 months at 1, 6, and 12 inch depths but poor survival at 24 inches. Young and Morris (111) reported that at least a 4-year rotation was necessary to grow sunflowers on a field with a history of *S. sclerotiorum*. Cook (28) also found that rotation of bean with corn and sugarbeets in a 3-year cycle was not effective to control SSR in Nebraska. Nevertheless, it was demonstrated that rapid decline of viable sclerotia of *S. sclerotiorum* and other sclerotium-producing fungi occurs in the field and that sclerotia surviving for long periods are only a small fraction of the original populations (6). Kurle et al. (57) showed that tillage affects sclerotia viability and eventually survival. Most viable sclerotia were found in the upper 2 cm of soil with non-tillage while viability was reduced in chisel plow and moldboard tillage. In contrast, in another study on lettuce drop incidence, viability of sclerotia was significantly higher following deep plowing (89). Coley-Smith and Cooke (27) discussed the factors that influence sclerotium survival. Such factors could be: structure (e.g., ring); environment (moisture, temperature, aeration, pH, soil organic matter and agricultural chemicals such as fungicides and herbicides); and biological agents (such as parasites and predators).

Sclerotium germination. Sclerotia exhibit dormancy that must be broken by preconditioning processes such as low temperature activation or thermocycling before they can germinate (27). Low temperature activation requires chilling sclerotia at 0-5 ^{0}C for 4-8 weeks and then incubating them at 16-24 ^{0}C (1,27,32). Some isolates of *S. sclerotiorum* do not require low temperature activation to germinate (50). Carpogenic germination requires the presence of free water and considerable energy, which is mobilized by conversion of food reserves (e.g. carbohydrates) in the sclerotium into soluble forms (27,60). Sclerotia of *S. sclerotiorum* can germinate eruptively (myceliogenically) by forming hyphae, or carpogenically by forming apothecia (3). Mycelial infection has been observed but it has been suggested that this rarely occurs under natural conditions (3). Steadman and Nickerson (84) demonstrated that carbohydrates can inhibit carpogenic but not myceliogenic germination. Similar results were reported by Bedi (11) for sclerotia incubated on nutrient media.

The mechanism of preconditioning is unknown but may be related to the age of the sclerotia (12). However, ageing alone does not explain why conditioning was more effective in soil than in moist vermiculite, although the possibility of microbial stimulation cannot be ruled out (78). Variations in incubation period are related to the sclerotia source; sclerotia from the field germinate sooner that those from pure cultures when suitable conditions are provided (1,66).

Light is required for development of fertile apothecia (27). It has been reported that mature apothecia can be developed after 5 days in near-UV light at 22 ^{0}C (70). Only light below 390 nm was effective in inducing apothecium formation (49). A comprehensive study of the light effect on apothecium formation was done by Ikegami (53) with *Sclerotinia trifoliorum* Erikss.

Apothecia production was observed after soybean canopy closure during mid July (15) and continued for a period of up to 5-6 weeks (15). Williams and Stelfox (106) found that farming practices had a significant effect on carpogenic germination of sclerotia. More sclerotia germinated in plots in which rapeseed followed rapeseed than in plots sown with rapeseed for only one year. Furthermore, germination was significantly higher in plots with surface-cultivation and spring fertilizer application than in plots of deep-plowing (106).

Pathogenesis. Lumsden (61) described the histology and physiology of pathogenesis in plant diseases caused by *Sclerotinia spp.* Some of the factors involved in the pathogenesis of *Sclerotinia spp.* are: (i) production of penetration structures (infection cushions or appressoria) that help in host cuticle penetration, (ii) formation of infection hyphae that develop between cells beneath the cuticle and in the cortex, (iii) synthesis of pectolytic enzymes and oxalic acid, which help in degrading the middle lamellae of host cells, and (iv) production of enzymes that hydrolyze cell wall and protoplasmic constituents and provide a steady supply of nutrients for rapid growth and development of infection hyphae.

Life cycle and symptomatology. Sclerotinia stem rot of soybean is monocyclic. The life cycle begins with the carpogenic germination of sclerotia. Ascospores released from mature apothecia are the source of primary infections (3,29,73). They are released by forcible discharge, which is triggered by a sudden change in relative humidity (46,62). Ascospores become airborne and alight on nonliving or senescent plant parts, after which they germinate, ramify the nonliving plant parts, and invade healthy plant parts by means of the mycelium that has developed from the ascospore (80). Senescing flower petals serve as an exogenous source of energy, which seems to be necessary for infection of non-injured healthy plants (3,15). It has been suggested that ascospores may penetrate healthy host tissue directly and establish infection, but this type of infection seems to be rare (80). White mycelial growth can be observed on the stems, leaves, and pods of infected plants when environmental conditions are favorable (80). Sclerotia are formed from the mycelia found inside or on the surface of the infected tissues (80). Eventually sclerotia reach the soil, where they remain on the surface or are buried (80). Apothecia are produced after sclerotia have been preconditioned or mycelium may develop, and thus a cycle is completed (80). Mycelial infection is considered a rare occurrence under natural conditions (3,29). Also, this type of germination is associated with small sclerotia (80).

The first symptoms on leaves or young stems are watersoaked spots that may enlarge and become a watery soft rot (80). Lesions are commonly observed on a main stem 15-40 cm above the soil surface and are also observed on lateral branches and on a main stem at the soil surface (40). Progressively, upper leaves wilt, become grayish green, and eventually turn brown (40). Fungal activity results in almost total destruction of parenchymatous tissues, and the remaining vascular and structural elements of stems, stalks, branches, and twings have a characteristic shredded appearance (40,80).

Epidemiology of *Sclerotinia* diseases. In beans, white mold epidemics are initiated by ascospores produced by sclerotia of *S. sclerotiorum* (1,2,28). Only sclerotia in the top 2-3 cm of the soil are functional, because apothecia with stipes longer than 3 cm are rarely produced under field conditions (3). Sclerotia present in and outside of bean fields also can provide ascosporic inoculum for bean white mold epidemics (3,100). Also, sclerotia have been observed to produce apothecia near host plants in hedge rows, uncultivated wooded areas, and fruit orchards (3).

Several factors are known to influence carpogenic germination of sclerotia; however, prolonged high soil moisture is the most common limiting factor (1,30,40,42). It has been shown that preconditioned sclerotia require matric potentials at or above -7.5 bars for about 10 days or longer to produce apothecia (42,66,95). However, apothecia may emerge after a period of intense rainfall even though drying may already have occurred (15,67). Hartill (45) did not find apothecia of *S. sclerotiorum* during prolonged dry periods, but apothecia appeared within 1 week of heavy rain. It has been suggested that sclerotia may be capable of continued carpogenic germination when exposed to brief periods of alternating high and low soil matric potentials (15).

Abawi and Grogan (3) suggested that temperature is an unlikely limiting factor for white mold epidemics in most bean-producing areas of the U.S. It was reported that irrigation (102) and amount of precipitation (71) influenced the severity of white mold but that Sclerotinia stem rot in the north-central region of the United States was predicted more accurately from air temperatures recorded before and after crop flowering (39). Also, in a recent 4-year survey conducted in the North-Central Region of the United States (107), it was concluded that summer air temperature rather than precipitation is the limiting factor for soybean Sclerotinia stem rot epidemics for these 4 years. Generally, exposure to extreme drying and possibly high temperature had a prolonged detrimental effect on apothecial production; however, sclerotia remained viable, as indicated by consistent mycelial production on nutrient media (3). Similarly, Philips (78) showed that sclerotia exposed to extreme drying and heating in soil for 5 years retained their capacity for carpogenic germination.

Information is available on the liberation, transport, and deposition of ascospores of *S. sclerotiorum* (91-93). With slight decreases in moisture tension, mature asci forcibly discharge ascospores into the air to a distance of more than 1 cm (46). This height of discharge enables the ascospores to escape the still layer of air near the soil surface and to reach the more turbulent aboveground layers (3). A mucilaginous material that can cement the spores to host tissues is discharged along with the ascospores. Ascospores deposited on bean tissues need not infect immediately, but can survive for a considerable time until wet conditions and exogenous energy sources required for infection are available (42). Blossoms serve as an energy source. Epidemics of white mold of beans occur only after flowering, although, a few infected plants were occasionally observed in fields prior to blossoming (3). Similar observations were reported by Boland and Hall (15) on soybeans Sclerotinia stem rot incidence.

Infection by *S. sclerotiorum* occurs only if free moisture is maintained for a relatively long time at the interface of tissues and the inoculum (1). Disease incidence was observed to be more severe with increased number of rain days (65). In controlled conditions, symptoms of white mold are developed after at least 54 hr of continuous plant surface wetness at 20 ^{0}C, but in field conditions the symptoms were observed after only 39 hr of plant surface wetness (15). Similar results have been reported from Lamarque (58) on flowering heads of sunflower. Once the epidemic starts, a number of new lesions of Sclerotinia stem rot appear with shorter plant surface wetness periods of 3-17 hr (13,15).

Epidemics are generally more common in fields with heavy vegetative crops and in areas where air circulation is limited, such as in low-level fields, particularly those surrounded by uncultivated areas (10,47). Generally, the disease cycle of Sclerotinia stem rot of soybean appears to be similar to that of white mold of white bean (15).

Mechanisms of dissemination. The means by which *Sclerotinia spp.* can be established or introduced, spread from field to field, and disseminated from one geographical area to another have been summarized by Adams and Ayers (5). Windblown ascospores are the major way for field-to-field spread (5,100), although sclerotia or mycelium contained in soil adhering to seedlings, farm equipment, animals, or man could be means of dissemination (5). Also irrigation runoff, the spreading of manure on fields (where diseased plant tissues are used as livestock feed), and seed infected by or surface-contaminated with mycelia or contaminated with sclerotia are possible means of dissemination (5,19,85). Probably the greatest potential for long distance dissemination of *Sclerotinia spp.* is either by seed infected with mycelia or by seed contaminated with sclerotia (5).

Within a field, dissemination is mainly through ascospores released from apothecia (3,15). Boland and Hall (16) concluded that SSR incidence is determined primarily by inoculum produced within a field. Also, it has been reported that the majority of ascospores are deposited close to a point source of apothecium inoculum, and the number of deposited apothecia decreases as the distance from the source increases; nevertheless, some spores may travel several hundred meters (91-93,100).

B. Control of *Sclerotinia* diseases

Steadman (86) discussed the methods used to control diseases caused by *Sclerotinia* species. The high pathogenicity of these fungi under favorable conditions, the ability of their sclerotia to withstand adverse conditions, and their wide range of hosts increase the difficulty of controlling these fungi (87). Control methods include: a) *Chemical control.* Chemicals should be applied before infection occurs. Foliar protectants, such as benomyl, PCNB, and DCNA have been reported to be effective when used in different numbers of spray applications among different areas of USA (52,86). In the case of beans, it has been demonstrated that the efficacy of fungicidal control of white mold depends on adequate coverage of blossoms with the chemical (52,86). In any case, chemical applications must precede the onset of the disease, so if epidemics could be predicted, the expense of routine fungicide applications could be reduced (86). Also, several sclerotium germination inhibitors and soil disinfectants, such as methyl bromide or formaldehyde, cyanamide, DCNA, and PCNB, have been effective in destroying sclerotia in the soil (86). Seed treatments with fungicides such as captan, benomyl, thiabendazole, and thiram can also be used to eliminate seed-borne *S. sclerotiorum* and prevent the dissemination of the fungus in new fields (108,110).

b) *Biological control.* More than 30 microorganisms have been identified that antagonize almost every stage in the life cycle of *S. sclerotiorum* (68). In the soil, antagonists inhibit sclerotial carpogenic germination (63), attack the hyphae produced during myceliogenic germination of the sclerotia (112), or attach the sclerotium itself (8,9,23,68). Several reports of studies on biological control of *S. sclerotiorum* are available (20,44,54,63,77,96,103,113). Some of these focused on screening mycoparasites on culture media under laboratory conditions (68,77,103). Some others have examined the effect of mycoparasites on SSR incidence in the field (20,44,63). However, only a few have examined the effect of environmental conditions on the activity of mycoparasites (44,77) or the interactions between different parasitic fungi (20). The potential for biological control of Sclerotinia diseases exists; however, more work is needed so that practical recommendations can be made.

c) *Management practices.* Crop rotation is recommended for control of Sclerotinia diseases. Williams and Stelfox (106) observed higher numbers of germinated sclerotia when rapeseed were planted in 2 consecutive years than when 1 year of rapeseed was followed by barley. However, they concluded that the number of germinated sclerotia did not diminish appreciably even by up to 3 consecutive years of barley following rapeseed, and fields infected with *S. sclerotiorum* can act as sites of inoculum production even after several years of rotation with nonhost crops (106). Similarly, in another study (81) on dry edible beans it was shown that a 3-year rotation did not reduce sclerotium populations significantly, probably because sclerotia survive in the soil at least 3 years. Deep plowing also has been recommended for control of white mold –at least during the year following plowing (106). No-tillage significantly reduces SSR occurrence (57,106,107). Fewer apothecia have been found in no-tillage (36,57). Kurle et al. (57) concluded that canopy density differences are more important to SSR incidence than are differences in numbers of viable sclerotia among tillage systems. Flooding has been reported to be an efficient method for destroying sclerotia of *S. sclerotiorum* in Florida although this technique would have limited usefulness in nonirrigated areas (64). Reduction in the number of irrigations, especially those at the end of the season, can reduce disease in the absence of rainfall. Also, populations of sclerotia are evenly redistributed in the upper soil profile of a field by irrigation water and thus the fungus is disseminated into low-lying areas of the field where microclimatic conditions may be favorable for disease development. These results come from studies conducted in Nebraska on irrigation frequency and mold development (10,83,102). Association of plant canopy development and Sclerotinia disease incidence has been observed in several crops, since light, soil temperature and soil moisture are critical factors for sclerotia germination and apothecia production (3,49,59,83).

Generally, an open plant canopy facilitates air circulation and light penetration and results in a more rapid drying of dew-covered leaf and moist soil surface (82). Effects of row spacing, growth habit, and plant density on canopy development, disease incidence and severity were examined in bean fields in Nebraska (30,83) and in potato fields in New York (75). Planting in wide rather than in narrow row widths has been suggested as a successful management practice of SSR control (83). Fertilizer treatments did not affect sclerotium germination (106), manure applications were positively correlated to SSR incidence (74) and increased carpogenic germination has been observed in soils with high organic matter content (33). All these types of treatments have a significant effect on canopy growth and plant standing.

d) *Resistant varieties.* Genetic resistance to *Sclerotinia spp.* was first observed by de Bary in 1887 (86). In beans, resistance has been observed in Nebraska and New York, but it was linked with late maturity (86). Reports before 1968 indicate that many researchers accepted the idea that resistance to *S. sclerotiorum* did not exist (86). Results from latter efforts in screening soybean cultivars for resistance to *S. sclerotiorum* have been variable, and results from greenhouse and field screening tests for the same varieties are usually inconsistent (25,37,39). Grau and Bissonnett (37) observed that the cv. Clay was susceptible in the greenhouse but resistant in field tests. Boland and Hall (14) found no correlation between greenhouse and field evaluations for Sclerotinia stem rot resistance. Nelson et al

(72) speculated that light differences might contribute to the lack of correlation between greenhouse and field screening results, and the importance of light was recently confirmed by Pennypacker (76). Grau et al (38) concluded that field resistance to soybean Sclerotinia stem rot is a heritable trait. A number of institutions and companies have attempted to incorporate resistance to *S. sclerotiorum* into snap beans, dry beans, and other crops but this has proved to be difficult because Sclerotinia stem rot resistance is difficult to identify consistently in the field due to disease escape (14,72). The combination of physiological resistance and canopy architectural avoidance theoretically is the best approach to improved, high-yielding, white-mold resistant varieties (14,87).

C. Relationship between yield and SSR incidence

Many approaches are available to quantify the disease-loss relationship. One is the use of empirical models derived from data generated from field experiments or surveys. Empirical models are usually divided into linear and non-linear models according to the statistical technique used (94). Few yield loss models are available in the literature for Sclerotinia stem rot, and all of them are linear regression single point models (25,48,109). These models have demonstrated that for every 10% increase in SSR incidence, soybean yield reduction should be expected to be between 147 and 263 kg/ha in Illinois (48), 235 kg/ha in Michigan (25) and between 170 and 330 kg/ha in Iowa (109). These results seem to be generally in agreement. Nevertheless, it is well established that in disease loss assessment studies (94) there are often great variations in the relationship of yield loss to disease incidence. In soybean, it has been suggested that these variations could be largely due to the spatial compensation capacity of the plants (108).

D. Predictive models

The science of epidemiology is concerned with describing and understanding disease at the population and community level (22). One way epidemiologists study populations is by using models, of which many different classifications exist. One way to classify models is based on how they are used (22). According to Campbell and Madden (22) a *predictive* or *forecasting* model (often also called a *disease-warning system*) is used to predict the likelihood of future disease increase based on a set of independent variables. Fry and Fohnet (34) defined a disease forecasting model as a predictive model that gives information about the probable occurrence or non-occurrence of plant diseases in economically important levels.

Forecasting models are classified based on the manner in which they were developed (35). Parameter values of a fundamental or mechanistic model are derived from experiments in a laboratory, growth chambers, greenhouses, or fields, and relate to one or more aspects of the host-parasite interaction with environmental conditions. An empirical system is developed from observations and analysis of current and historical data on disease development, weather conditions, and other biotic and abiotic conditions. Empirical systems may be qualitative (e.g., Stevens'system for Stewart's bacterial wilt) (88) or quantitative (e.g., the regression model for Septoria leaf blotch severity of wheat based on rainfall and temperature) (26).

Fry and Fohner (34) summarized some of the objectives for developing forecasting systems, such as increasing farm income by providing more efficient allocation of disease management resources; reducing the risk of large losses in crop value from disease; and reducing pesticide applications and thus reducing potential harmful effects on the environment and on human health. Generally, forecasting systems have found application in disease management. Their use is likely to increase when they are incorporated into crop-management systems (112).

Forecasting systems are useful in some situations, but not in others. They are appropriate for those diseases that occur sporadically (18). If a disease is always important, management efforts will be required, and thus the forecast provides useful information in optimizing the timing of fungicide applications. If a disease is never important, predicting it is unnecessary (18). Effective technology for disease suppression and mechanisms to distribute the results of forecasts are necessary to justify the development or use of a forecast (18).

The elements of a successful forecasting system, summarized by Campbell and Madden (22), are: reliability; simplicity; importance of the forecast disease; usefulness; availability; and cost effectiveness. Most of the developed forecasting systems have failed to meet one or more of these elements. This may be one of the main reasons why forecasting systems have limited application even though a large number of forecasting models are available in the literature.

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CHAPTER III. EFFECTS OF FLUCTUATING TEMPERATURE AND WATER POTENTIAL ON SCLEROTIUM GERMINATION, APOTHECIUM PRODUCTION AND FUNGI ISOLATED FROM THE SURFACE OF SCLEROTIA OF *SCLEROTINIA SCLEROTIORUM*

A paper prepared for Phytopathology

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A. Abstract

The effects of fluctuations in soil temperature and soil water potential on sclerotia germination, apothecium production and fungal species isolated from the surface of sclerotia of *Sclerotinia sclerotiorum* were investigated. For the temperature experiments, four daily temperature fluctuations (18-22, 16-24, 14-26, and 12-28 °C), which had the same daily mean temperature of 20 °C, and a constant temperature (20 °C) treatment were used. For the soil water potential experiments, a constant saturation treatment ($\psi_m \cong$ -0.01 bars) and three soil water matric potential (ψ_m) fluctuations were used. Soil water matric potential fluctuation treatments were generated by drying the soil until ψ_m reached the values of -0.3 to -0.4 bars (high ψ_m), -0.6 to -0.7 bars (medium ψ_m), and -0.9 to -1.0 bars (low ψ_m). Daily temperature fluctuations of 4 or 8 °C increased sclerotium germination and apothecium production compared to fluctuations of 12, 16 °C and the constant temperature treatment. The first apothecium appeared in the 8 °C fluctuation treatment 24 days after the experiment initiation. Sclerotia in the 12 °C fluctuation germinated last, 44 days after the experiment initiation. Moisture fluctuations were detrimental to sclerotium germination and apothecium production. Constant saturation yielded the highest number of germinated sclerotia and apothecia. The first sclerotium in the saturation treatment germinated 35 days after the

experiment initiation, and after 76 days in the low matric potential fluctuation. In the temperature experiments, *Fusarium* spp. were isolated frequently from sclerotia in the low temperature fluctuation treatments (0, 4, and 8 °C) and the only fungi isolated from the high temperature fluctuation treatments (12 and 16 °C). In the moisture experiments, xerophytic species such as *Penicillium* spp., and *Fusarium* spp. were isolated frequently from all soil water potential fluctuations. Species such as *Gliocladium* spp., *Coniothyrium* spp., and *Alternaria* spp., known as antagonists of *S. sclerotiorum*, were recovered at low frequencies.

B. Introduction

The host range of Sclerotinia stem rot (SSR) of soybean, caused by *Sclerotinia sclerotiorum* (Lib.) de Bary, includes more than 360 species in 62 plant families (31). Before 1990, in the North-Central soybean production region of the USA, Sclerotinia stem rot was causing localized epidemics in Michigan, Minnesota, and Wisconsin (17,18,19). Since 1990, outbreaks of SSR have become more frequent and severe (12,42,43) in the North-Central soybean production region. Practices designed to increase soybean yield, such as narrow row spacing, high plant density and early planting dates are associated with the recent outbreaks of SSR in the North-Central Region (25).

Sclerotinia sclerotiorum survives in the soil for long periods as sclerotia. SSR epidemics are initiated by ascospores released from apothecia formed by sclerotia. Apothecia with stipes longer than 3 cm rarely are formed under field conditions (2). Mycelial infection of plant tissues has been observed but occurs rarely under natural conditions (2). Thus, ascospores are considered to be the primary source of inoculum.

Carpogenic germination of sclerotia decreases with depth of burial in soil. Radulescu and Crisan (32) showed that germination was the highest at a soil depth of 2-5 cm. In another study (10) the majority of sclerotia that formed initials in the soil were in 5 and 10 cm depths. Williams and Western (41) found that apothecia were formed down to 5 cm but in diminishing numbers with increasing depths. Among the environmental factors affected by burial depth are aeration and moisture (30). At shallow depths, moisture and temperature fluctuate widely and the soil dries out frequently. At greater soil depths, where moisture and temperature levels are more stable, length of apothecium stipes becomes the limiting factor for sclerotia germination since produced apothecia initials cannot reach the soil surface, and thus release ascospores (30).

Several factors are known to affect carpogenic germination of sclerotia of *S. sclerotiorum* (9). Soil moisture is a common limiting factor (1,9). Prolonged periods of low soil temperature and high soil moisture are favorable for apothecial development (20). Continuous saturation is required for apothecial development and even a slight moisture tension prevents apothecial formation (2). Duniway et al. (13) demonstrated that formation of initials is stopped at matric potential -0.08 to -0.38 bars, while there is no growth of mature apothecia at matric potentials below -0.5 bars.

Fewer apothecia have been reported beneath an open than a dense compact bean canopy (33). A dense canopy is common in narrow planting rows, where SSR severity was greater than in wide rows (18,36). Tillage practices were also associated with SSR incidence, since fewer dead plants and apothecia were found in no-till than plowed soybean fields (25). In another study, SSR was found more prevalent in fields under minimum till than no-till or conventional tillage (42). It was suggested that in no-tillage sclerotia remain on the soil surface and are exposed to dry conditions, whereas in minimum tillage, sclerotia are buried at shallow soil depths where moisture and temperature levels are more stable and apothecium stipes may reach the soil surface (42).

Numerous microorganisms have been identified as antagonists at almost every stage in the life cycle of *S. sclerotiorum*. In the soil, antagonists inhibit sclerotial carpogenic germination (27), attack the hyphae of myceliogenic germination (44), or colonize the sclerotium itself (3,4,7,27). Some of the studies on screening mycoparasites have been done on culture media and laboratory conditions (27,29,40) and others have directly examined the effect of mycoparasites on SSR incidence in the field (6,22,26). No studies have found on the interactions among different parasitic fungi and their synergistic (or antagonistic) activity on biological control of *S. sclerotiorum*, except for one on *Coniothyrium minitans* and *Gliocladium virens*, which found no strong synergism between the two species (6).

A few studies have examined the effect of different environmental conditions on mycoparasite activity (3,38). There is evidence that small changes in soil matric potential may influence the growth of soil-borne fungi, possibly by altering the populations and activity of their antagonistic fungi and bacteria (14). Smith (35) demonstrated that sclerotia of *S. sclerotiorum* returned to moist soil after being air-dried for 4 hr leaked amino acids and sugars, germinated and were decomposed within 2 weeks by mycoparasites. Smith suggested that germination of fungi, such as *Trichoderma* species that colonize and decompose sclerotia, appears to be stimulated by the nutrients leaking from sclerotia (35).

Soil moisture and temperature vary considerably especially in the top 2-3 cm of soil, and are affected by wind velocity, type and density of plant canopy and management practices such as tillage and row spacing. Buchan (5) and Campbell (8) observed high (up to 15 °C) daily temperature fluctuations on the bare soil surface that diminish rapidly within the top 8 cm of soil depth. Workneh and Yang (*unpublished data*) observed that soil temperature and moisture varied more on the soil surface than at 5 cm depth and with wide (30 cm) than narrow (15 cm) soybean planting rows. They observed that the coolest and wettest soil was at 5 cm depth in plots with narrow row planting, and the warmest and driest soil was at the soil surface with wide row planting. It is unknown how soil water potential and temperature fluctuations affect *S. sclerotiorum* sclerotial germination, apothecia production, and mycoparasitic populations. Objectives of the present study were to determine the effects of fluctuations in soil temperature and soil water potential on sclerotium germination, apothecium formation, and on frequency of fungi isolated from the surface of sclerotia. Frequency was determined as the percentage of sclerotia from the surface of which each fungal species was isolated.

C. Materials and methods

Sclerotia selection and preconditioning. Sclerotia were collected in late September of 2000 from a soybean field planted to PB239792 near Ackley, Iowa that had been severely infested with Sclerotinia stem rot. Sclerotia were sorted by size; large sclerotia (approximately 12-14 mm x 2 mm), were used in the experiments. Sclerotia were stored in paper bags at room temperature (approximately 20 °C) in the dark until they were used.

Before each experiment, sclerotia were tested for germination and then preconditioned. Fifty sclerotia chosen at random were hydrated for 30 min in sterile distilled water and plated individually on potato-dextrose agar (PDA) containing 0.15 mg of tetracycline and 0.15 mg of streptomycin per milliliter of PDA. Germination was evaluated 7 to 10 days after incubation at 20 °C on a 12-hr light-dark cycle (37). For preconditioning, 30 to 40 sclerotia were placed on a layer of sterile paper towel saturated with 5 ml of sterile distilled water in a sterile plastic Petri plate. The plates then were sealed and placed in a refrigerated incubator (5 to 6 °C) in darkness for eight weeks. In each experiment, preconditioned sclerotia were mixed to eliminate any differences in preconditioning among plates (37).

Temperature fluctuation experiments. Five daily temperature fluctuation ranges (0, 4, 8, 12, and 16 °C) were used. To generate ranges, four daily soil temperature fluctuations (18-22, 16-24, 14-26, and 12-28 °C, minimum at midnight and maximum at noon) and a constant temperature (20 °C) treatment were used. All treatments had average daily temperature equal to 20 °C (Fig. 3.1). Each treatment had three replicates. Each replicate consisted of three 31 x 23 x 11 cm crispers each containing 3 kg of sterilized sand. Sixteen preconditioned sclerotia were placed on the sand surface in each crisper. Crispers were placed in growth chambers (PGW36/Conviron) with temperature set to the lowest temperature group of crispers. The temperature in the crispers was controlled with stepless temperature controllers (Thermolyne Type 45500) and heating tapes around the crispers (Thermolyne/Briskheat 122 x 1.3 cm, 104 w). Soil temperature was constantly recorded with a data logger (CR10, Campbell Scientific, Inc). Light intensity in the growth chambers was set to be between 160 and 190 $molm^{-2}s^{-1}$ (at the level of the crisper covers) and at a 12-h light dark cycle. Sand in all crispers was kept near saturation (soil matric potential, ψ_m = -0.01 bars) for the duration of the experiment. Experiment was conducted four times (referred to as Experiment 1 through 4).

Moisture fluctuation experiments. Four treatments of soil water matric potential (SWP) ψ_m, were used: three fluctuating treatments and a constant saturation treatment. The fluctuation treatments differed in intensity and duration. Each treatment had three replicates.

Each replicate consisted of three 31 x 23 x 11 cm crispers containing 3 kg of sterilized sand. Sixteen preconditioned sclerotia were placed on the sand surface in each crisper. Initially all crispers were kept wet for 3 days. Subsequently, sand moisture was manipulated by drying the sand until ψ_m reached -0.3 to -0.4 (high water potential ψ_m), -0.6 to -0.7 (medium water potential ψ_m), and -0.9 to -1.0 (low water potential ψ_m) bars. Sand drying was achieved by removing crispers lids. When ψ_m for a given moisture treatment had reached its final value, the crispers belonging to this treatment were watered until the sand was saturated ($\psi_m \cong -0.01$ bars) (Fig. 3.2). Crispers were placed in growth chambers (PGW36/Conviron) with temperatures set at 20 °C. Sand temperature and matric potential ψ_m were monitored continuously in one crisper per treatment with Watermark soil moisture blocks (Campbell Scientific; sensor models 257). Watermark blocks are accurate between 0 and -2 bars. Light intensity in the growth chambers was arranged to be between 160 and 190 $molm^{-2}s^{-1}$ (at the level of the crisper covers) and at a 12-h light dark cycle. Experiment was conducted three times (referred to as Experiment 5 through 7).

Isolation and identification of parasitic fungi. At the end of each temperature and moisture experiment, sclerotia were plated on cornmeal agar (CMA) at 20 °C with a 12-h photoperiod for 7 to 10 days. At the end of this period, each sclerotium was examined at 30x with a stereomicroscope. Fungi growing on the surface of the sclerotia and the medium were transferred to APDA and incubated for 7 to 10 days at 20 °C with a 12-h light dark cycle for identification. The same procedure was followed for the preconditioned sclerotia that had not been used in the temperature and moisture experiments (total of 7 experiments for temperature and moisture) and for stored (non-preconditioned) sclerotia. In the case of stored sclerotia, the procedure was repeated two times (after 9 and 17 months in storage). Fifty sclerotia were used each time.

Data collection and statistical analysis. Experiments were checked every three days for the first apothecium appearance. Data were collected every two days after first apothecium appearance in a crisper. The number of apothecia and day of appearance were recorded. After recording, apothecia were removed to prevent counting duplications. Each experiment was conducted for 90 days. Linear regressions of total number of germinated sclerotia and apothecia against days after experiment initiation for each treatment were fitted to pooled data from all experiments. Beforehand, slopes of individual experiments were tested for heterogeneity to determine whether regression coefficients were significantly different from each other ($P<0.01$). Treatment was used as an indicator variable (with treatment of constant temperature and continuous saturation used as the reference groups for the temperature and moisture experiments respectively). This statistical analysis is useful in detecting quantitative effects of variables with discrete levels (28). Number of produced apothecia and day of first apothecium initiation were analyzed by one-way analysis of variance (PROC ANOVA). Mean separation was conducted using Duncan's multiple range test. Effects of temperature and water potential fluctuations on frequency of fungi isolated from sclerotia surface was tested by analysis of variance. All analyses were performed with SAS (version 8.1, SAS Institute, Inc., Cary, NC).

D. Results

In six of the seven experiments, myceliogenic germination was 100%. In one case, germination was 98%. Carpogenic germination of sclerotia was generally low. In the treatment of constant saturation and 20 °C, common in all seven experiments, carpogenic germination ranged between 25% (first experiment beginning in October of 2000) and 45% (sixth and seventh experiments beginning in March of 2001).

Temperature fluctuation experiments. Small temperature fluctuations (4 and 8 ^{o}C) increased apothecium production comparing to the large fluctuations (12 and 16 ^{o}C) and the constant temperature (Fig. 3.3A). The highest number of produced apothecia was observed in the 8 ^{o}C fluctuation (Fig. 3.3A). Rate of increase in produced apothecia over time was highest for 8 ^{o}C fluctuation, followed by the 4 ^{o}C fluctuation (Fig. 3.3B, and coefficient estimates for Days in Table 3.1). The number of germinated sclerotia and apothecia with 12 ^{o}C daily temperature fluctuation was similar to the constant temperature treatment, suggested by the estimated regression coefficients (Table 3.1). Apothecium production initiation was earlier at 8 ^{o}C fluctuations than in 0, 4, 12 and 16 ^{o}C (Fig. 3.4). The first sclerotium germinated in the 8 ^{o}C fluctuation treatment 24 days after the experiment initiation whereas the first sclerotium germinated in the 12 ^{o}C fluctuation treatment 44 days after the experiment initiation (Fig. 3.4).

Moisture fluctuation experiments. The constant saturation treatment had the highest number of germinated sclerotia and apothecia (Fig. 3.5A). The moisture fluctuation treatments significantly reduced the number of germinated sclerotia and apothecia comparing to the constant saturation treatment (Fig. 3.5). The largest reduction occurred in the low soil matric potential fluctuation (-0.9 to -1.0 bars) treatment, although this reduction was not significantly different from these of the high and medium soil matric potential fluctuations. The estimated coefficient of Days for the saturation treatment was the highest followed by these estimated for the high and medium water potential fluctuation treatments. The estimated coefficient for low soil matric potential fluctuation was the lowest (Table 3.2).

Sclerotia germination and apothecium production occurred earlier in the saturation treatment than in any fluctuation treatments. The first sclerotium in the saturation treatment germinated 35 days after the experiment initiation, and in the low soil matric potential fluctuation treatment sclerotia germinated last, 76 days after the experiment initiation (Fig. 3.6).

Isolation and identification of parasitic fungi. The frequency and number of fungi isolated from the sclerotia surface differed between temperature and moisture fluctuation experiments. In the temperature fluctuation experiments *Fusarium* spp. were isolated frequently from sclerotia in the low temperature fluctuation treatments and were the only species isolated from the two large range fluctuations (12 and 16 °C) (Table 3.3).

In the soil water potential fluctuation experiments, *Fusarium* spp. were recovered at high frequency from the saturation and high soil water potential treatments but significantly less often from the medium and low soil water potential treatments (Table 3.4). In the moisture fluctuation experiments the predominant species in medium and low SWP were *Penicillium* spp. (Tables 3.3 and 3.4).

In the temperature experiments, *Gliocladium*, *Coniothyrium*, and *Alternaria* spp., were recovered in lower frequencies than species such as *Fusarium*, *Penicillium*, and *Trichoderma* spp. and only from 3 treatments (0, 4, and 8 °C) (Table 3.3). In the 4 and 8 °C fluctuations the recovering frequencies of these species were lower than the ones at 0 °C, while *Fusarium*, *Penicillium*, and *Trichoderma* species were recovered in frequencies equivalent to the ones at 0 °C. In the soil matric potential fluctuation experiments, only *Gliocladium* spp. were recovered (Table 3.4). Recovery of *Gliocladium* spp. was similar in saturation and medium water potential fluctuation treatments and significantly lower in the high water potential fluctuation. *Gliocladium* spp. were not recovered from the low water potential fluctuation treatment (Table 3.4).

Fusarium spp. was the only species isolated from the surface of preconditioned sclerotia that were not used in the temperature and moisture fluctuation experiments. *Fusarium* spp. and *Penicillium* spp. were the predominant species isolated from the surface of sclerotia after 9 and 17 months in storage, while *Alternaria* spp. and *Gliocladium* spp. were also recovered in low frequencies (Table 3.5).

E. Discussion

Although it has been speculated that soil temperature and moisture fluctuations should have an effect on sclerotia germination (2) to our knowledge this epidemiological aspect has not been examined for *Sclerotinia sclerotiorum* or other species of Sclerotiniaceae. Our results demonstrate that fluctuations of soil temperature and soil water potential affect the subsequent carpogenic germination of sclerotia of *Sclerotinia sclerotiorum*. Whereas sclerotia germination and apothecia production occurred in a wide range of soil temperature and moisture fluctuations, the proportion of sclerotia that germinated and produced apothecia was greater under low temperature fluctuations and continuous moisture than the other temperature and moisture treatments.

Sclerotia germination and production of apothecia increased when the temperature fluctuations increased from 4 to 8 °C but decreased rapidly when the fluctuation range increased further to 12 and 16 °C. These results suggest that a range of 16-24 °C should be optimal for carpogenic germination. However, the exact maximum detrimental range could not be determined as a few sclerotia did germinate even in the most extreme fluctuation range of 16 °C. An important design of the temperature fluctuation experiments was that the average daily temperature was the same for all treatments (20 °C). Thus, our results demonstrated that, even if the average daily temperatures are the same, sclerotial germination and apothecial production are affected by temperature fluctuations.

Duniway *et al.* (13) demonstrated that production of apothecia of *Sclerotinia sclerotiorum* was very sensitive to soil matric potential, with the number of initials and mature apothecia being maximal at -0.08 to -0.38 bar and the growth of mature apothecia being prevented at matric potentials below -0.5 bar. Phillips (30) reported that sclerotia required free water or water potentials approaching 0 kPa for germination to take place. Abawi and Grogan (2) found that apothecia were formed at 0 kPa but not at -600 kPa. Also the same authors observed that continuous moisture for about 10 days was required for sclerotia germination and thus speculated that even slight osmotic stress inhibited germination. Nevertheless, soil matric potential was constant in each of these studies. The present study is the first report of the effect of soil matric potential fluctuations on *S. sclerotiorum* sclerotia germination.

Our results also suggest that optimum soil water potential condition for sclerotia germination and apothecia production is saturation ($\psi_m \cong -0.01$ bars) (Fig. 3.5). Soil water potential stress was detrimental to sclerotia germination and, apothecia production (Fig. 3.5). Even in the low soil water potential fluctuation treatment, the number of germinated sclerotia and produced apothecia was reduced significantly compared to the constant saturation treatment (Table 3.2). With continuous saturation sclerotia germinated significantly earlier than with any other soil water potential treatment, and had a duration of 60 days of regular apothecia production. It has been reported that sclerotia subjected to extreme drying at the soil surface did not form apothecia when subsequently put in ideal conditions though they were still viable (1). In our study, 12% of the apothecia in the soil water potential fluctuations (total of 3 experiments) were produced during the drying period of the treatment. Thus, during short periods of drying (1 to 4 days) sclerotia may still be able to produce apothecia.

In the 16 °C temperature fluctuation treatment, 15 sclerotia (7.8% of the total) germinated and produced 122 apothecia. In the high soil water potential treatment, only 2 sclerotia (1.4% of the total) were germinated and produced 5 apothecia. Although these results are not directly comparable, it seems that between the two worst scenarios (i.e. high soil temperature fluctuation with daily average of 20 °C but constant saturation, or high soil water potential fluctuation but constant temperature of 20 °C) the soil water potential fluctuation is more detrimental to the sclerotium germination and apothecium production than the temperature fluctuation.

Gracia-Garza et al. (16) observed a higher number of apothecia with reduced tillage than no tillage and suggested that this could be triggered by the higher soil moisture and nutrient availability, and lower soil temperature associated with reduced tillage than no tillage. Such differences in soil temperature and moisture have also been observed by Workneh and Yang (*unpublished data*) in combinations of different soil depths and row width. Our results support the generalization that minimum tillage and narrow soybean planting would provide more favorable environmental conditions than no tillage and wide row planting for sclerotia germination and apothecia production (16,25,42).

Fusarium spp. was the predominant species in the low temperature fluctuation treatments and the only species isolated from the high fluctuation treatments. There are a few reports (34,44) on antagonistic activity of *Fusarium* spp. on *S. sclerotiorum*, though the optimum environmental conditions for their development and activity on sclerotia have not been investigated.

Trichoderma and *Penicillium* species were recovered in high frequencies while *Coniothyrium* spp., *Gliocladium* spp., and *Alternaria* spp., that have been tested as biological control agents against *S. sclerotiorum* (6,22,29,40), were recovered in low frequencies from the temperature fluctuation treatments. Previous studies (23,29,39) reported that *Trichoderma viride* and *Penicillium* sp. are more effective at 20 to 25 $^{\circ}$C, while *Gliocladium* spp. has an optimum growth around 30 ^{0}C and *Coniothyrium* spp. around 15 to 20 $^{\circ}$C. Differences in optimum temperatures may account for the differences in fungal populations observed in our experiments.

We are not certain for the reasons leading to the dominance of the *Fusarium* species in temperature fluctuation experiments. Several fungi were isolated from the surface of stored sclerotia while only *Fusarium* spp. was isolated from the preconditioned ones for 8 weeks at 5 $^{\circ}$C sclerotia surface. Thus, it could be that *Fusarium* spp. survive better than other parasitic fungi at 5 $^{\circ}$C and then are able to be established earlier and faster on the sclerotia surface, obtaining sites that eventually might have been taken by other parasitic fungi.

In the moisture fluctuation experiments, numbers of isolated fungi were similar in constant saturation and high matric potential treatments. In the medium and low matric potential fluctuation treatments there was a reduction in the frequencies of isolated *Trichoderma* spp. and *Fusarium* spp. and an increase on the frequencies of *Gliocladium* spp. and *Penicillium* spp. For *Gliocladium* spp., this result is in agreement with a previous study (29) in which the percentage of sclerotia infected by *Gliocladium virens* was higher with low than with high soil moisture content. Also, for *Trichoderma* spp. a study (23) reported that incidence of sclerotia colonization by *T. harzianum* was higher in -0.5 than -0.05 bars.

Moreover, it was reported (15,24) that as the water content declines gradually and takes values below -1.4 to -3.8 bars *Penicillium*, *Aspergillus*, *Fusarium*, and *Trichoderma* spp. become predominant. We also took similar observations, for *Penicillium*, and *Fusarium* species. Griffin (21) reported that interspecific antagonism between fungi in soil limits the activity of most species to water potentials higher than those which they can tolerate in pure cultures. A few fungi, mainly xerophytic species of genera *Penicillium*, *Aspergillus*, and some *Fusarium* species grow almost equally well in pure and mixed cultures at low water potential, due to the greatly reduced antagonism from the general microflora (21).

With no tillage, in the upper layer of soil, populations of microorganisms are double than in conventional tillage (11). Gracia-Garza et al. (16) suggested that reduced number of produced apothecia observed in no tillage could be due to the activity of soil microorganisms affecting the germination and survival of sclerotia. We draw the attention that the fungal frequencies reported in the present study may be the results of pre-selection during storage or sclerotia preconditioning and incubation conditions. It would be useful, however, to examine the activity of mycoparasites in combination with different production practices such as tillage and row spacing, since these practices are associated with alteration of the soil microclimate, the area of mycoparasitic activity.

F. Acknowledgement

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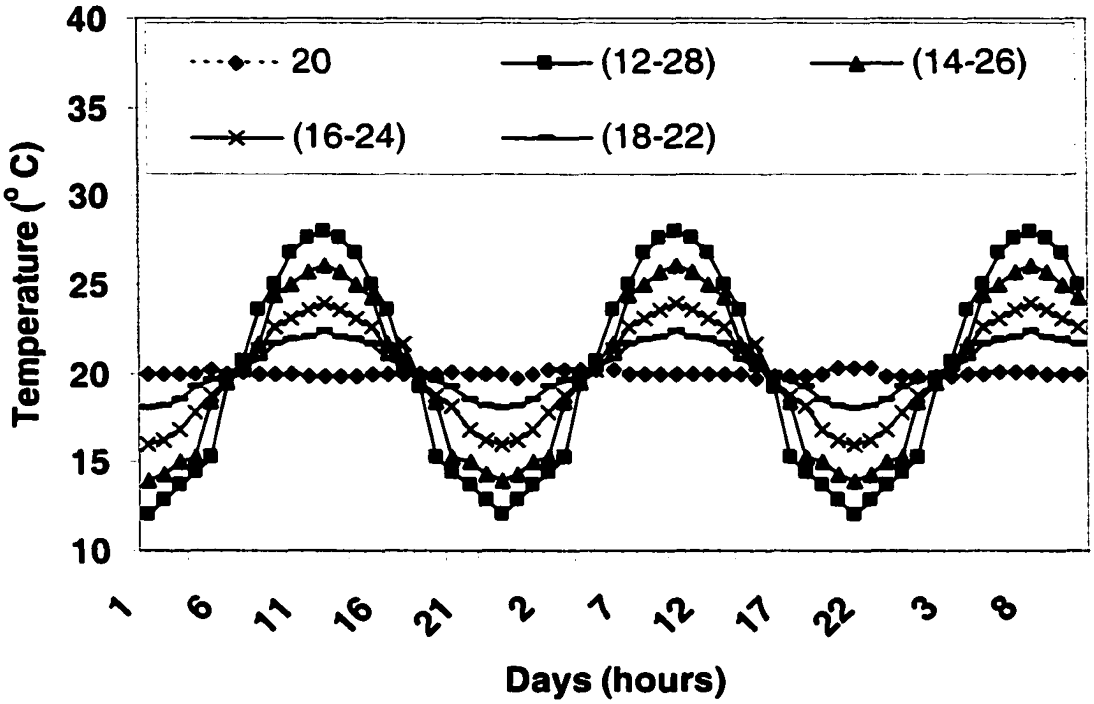


Fig. 3.1. Two and a half days (in hourly intervals) of the four soil temperature fluctuations (18-22, 16-24, 14-26, and 12-28 °C) and the constant temperature (20 °C) used in the temperature experiment. Maximum and minimum of the fluctuations were at noon and midnight, respectively. Temperature fluctuations were generated using stepless temperature controllers (Thermolyne Type 45500) and heating tapes (Thermolyne/Briskheat 122 x 1.3 cm, 104 w). Temperature was constantly recorded with a data logger (CR10, Campbell Scientific, Inc).

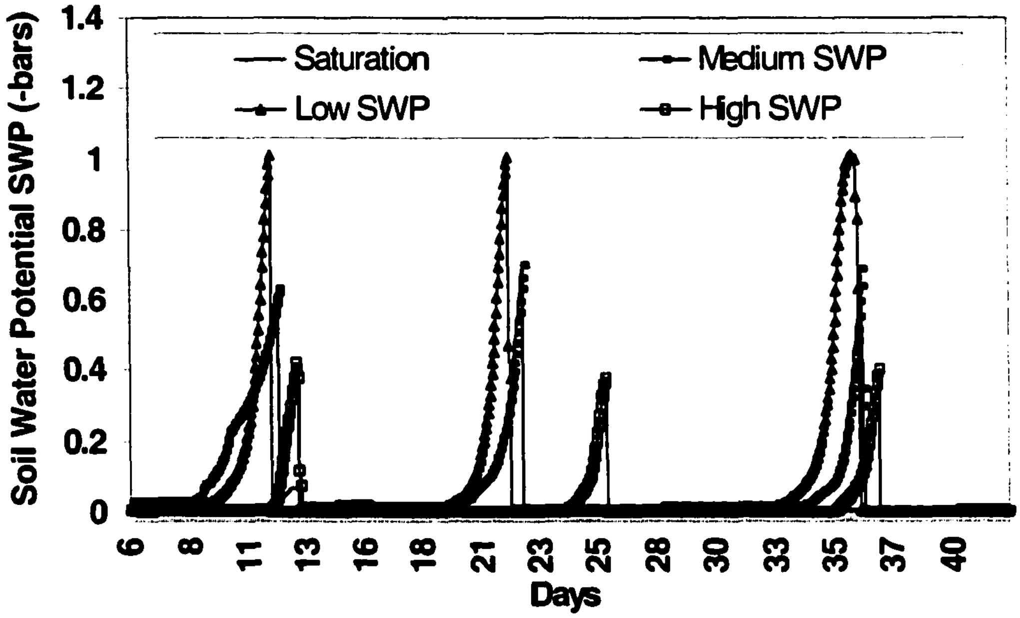
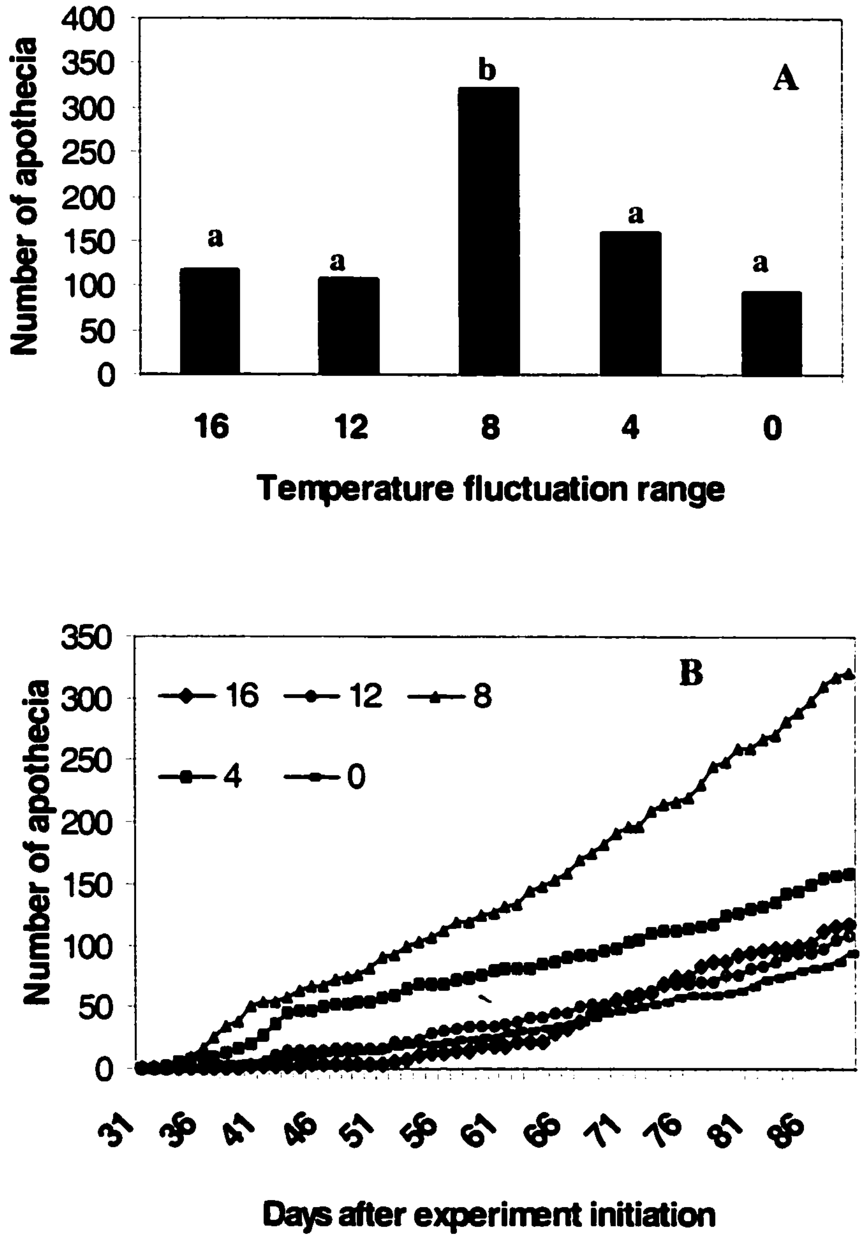


Fig. 3.2. Soil water matric potential (SWP), ψ_m fluctuations used in the soil moisture experiments. SWP (ψ_m) was manipulated by drying the soil until ψ_m reached the values of -0.3 to -0.4 bars (high water potential), -0.6 to -0.7 bars (medium water potential), and -0.9 to -1.0 bars (low water potential). When ψ_m for a given fluctuation treatment had reached its final value, water was added to the crispers of the treatment until soil was saturated ($\psi_m \cong$ -0.001 bars). SWP was measured continuously with soil moisture blocks (Watermark blocks, Campbell Scientific; sensor models 257) and constantly recorded with a data logger (CR10, Campbell Scientific, Inc).

Fig. 3.3. Total number of apothecia produced during the temperature experiment **(A)**, and rate of apothecia production **(B)** observed at 0, 4, 8, 12, and 16 °C fluctuations (generated with 20-20, 18-22, 16-24, 14-26, and 12-28 °C daily temperature fluctuations respectively with maximum temperature at noon and minimum at midnight). Forty-eight sclerotia were used in each treatment. Numbers of apothecia are means of four experiments. Columns with the same letters are not significant different from each other by Duncan's multiple range test ($P = 0.05$).


400
350
300
250
200
150
100
50
0
Number of apothecia
b
A
a
a
a
a
16
12
8
4
0
Temperature fluctuation range
350
300
250
200
150
100
50
0
Number of apothecia
B
16
12
8
4
0
31
36
41
46
51
56
61
66
71
76
81
86
Days after experiment initiation

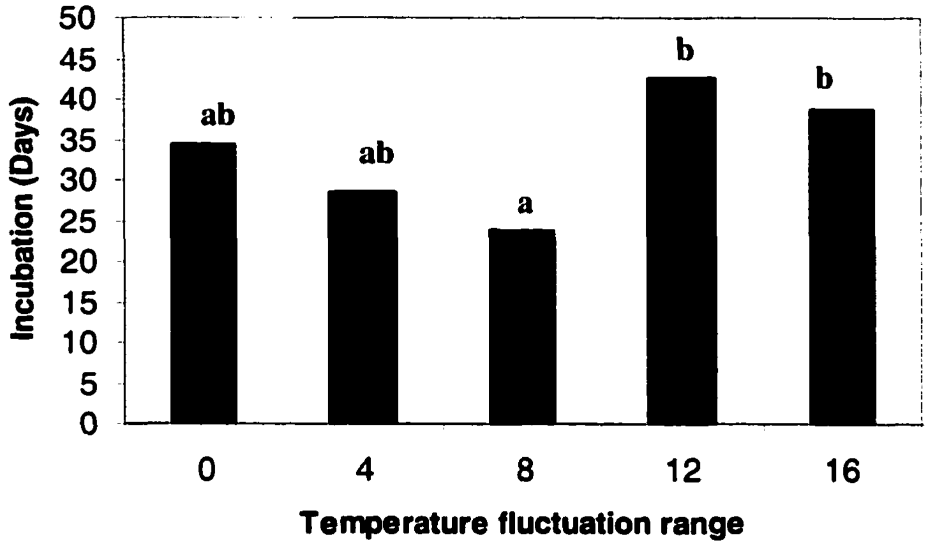
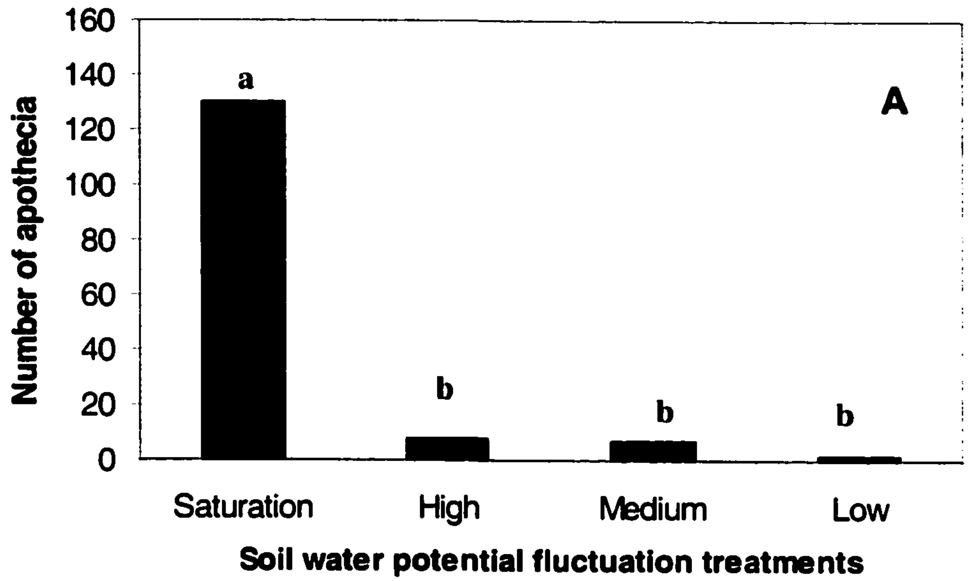
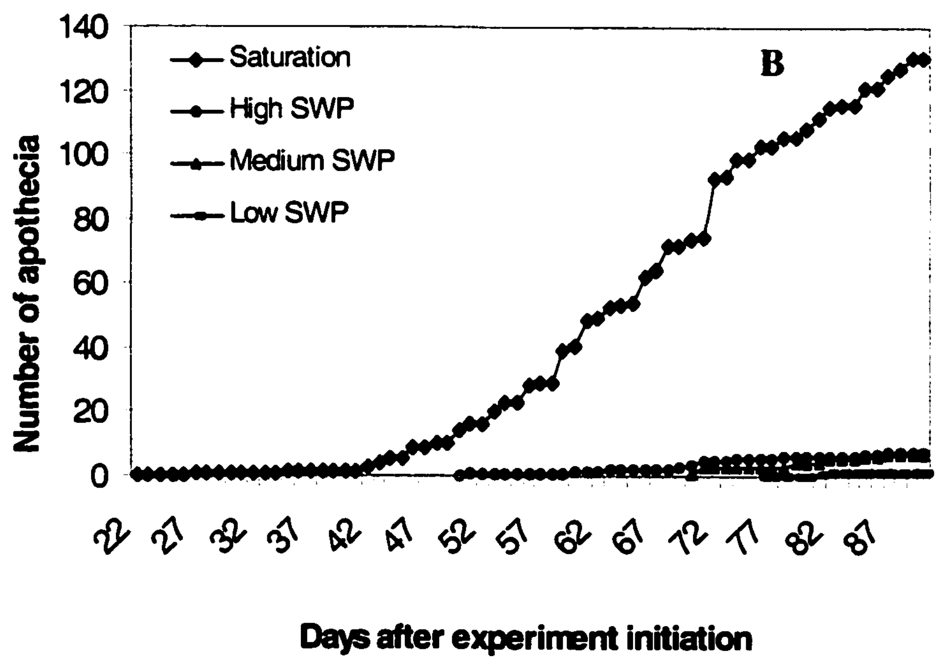


Fig. 3.4. Time (days) from the start of the experiment until the first apothecium appearance in the temperature fluctuation treatments of 0, 4, 8, 12, and 16 °C (generated with 20-20, 18-22, 16-24, 14-26, and 12-28 °C daily temperature fluctuations, respectively, with maximum temperature at noon and minimum at midnight). Bars are means of four experiments. Means with the same letters are not significantly different from each other by Duncan's multiple range test ($P = 0.05$).

Fig. 3.5. Total number of apothecia produced during the moisture experiment **(A)**, and rate of apothecia production **(B)** observed at saturation (≅ -0.01 bars), low (-0.9 to -1.0 bars), medium (-0.6 to -0.7 bars), and high (-0.3 to -0.4 bars) fluctuations of soil water potential (SWP). Forty-eight sclerotia were used in each fluctuation treatment. Numbers of apothecia are means of three experiments. Columns with the same letters are not significant different from each other by Duncan's multiple range test ($P = 0.05$).


160
140
120
100
80
60
40
20
0
Number of apothecia
a
b
b
b
A
Saturation
High
Medium
Low
Soil water potential fluctuation treatments


140
120
100
80
60
40
20
0
Number of apothecia
Saturation
High SWP
Medium SWP
Low SWP
B
22
27
32
37
42
47
52
57
62
67
72
77
82
87
Days after experiment initiation

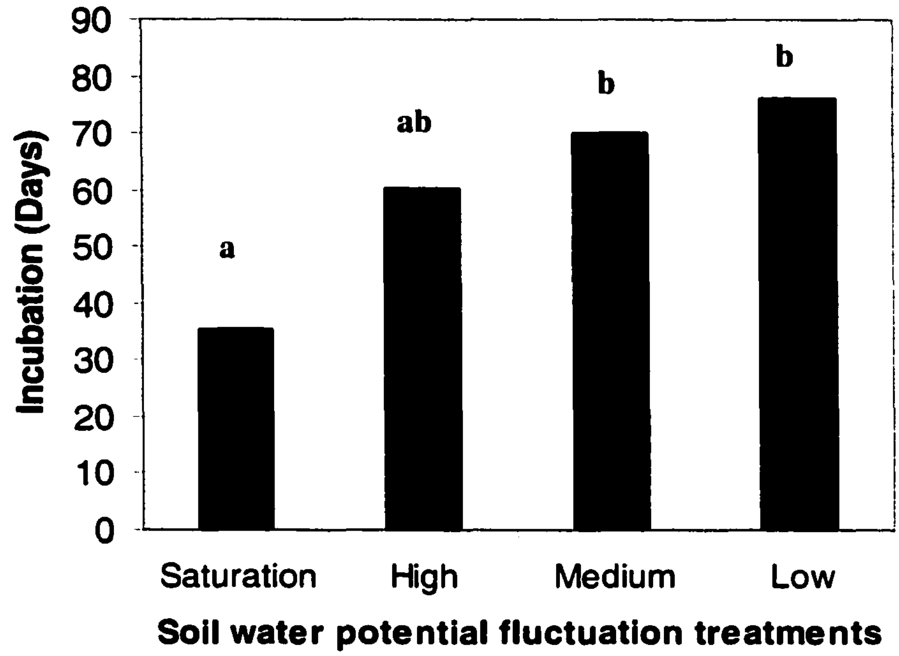


Fig. 3.6. Time (days) from the start of the experiment until the first apothecium appearance in the soil water potential (ψ_m) fluctuation treatments of saturation ($\equiv$ -0.01 bars), high (-0.3 to -0.4 bars), medium (-0.6 to -0.7 bars), and low water potential (-0.9 to -1.0 bars). Bars are means of three experiments. Means with the same letters are not significantly different from each other by Duncan's multiple range test ($P = 0.05$).

Table 3.1. Parameter estimates of linear regression of total number of germinated sclerotia and apothecia of *Sclerotinia sclerotiorum* with number of days after experiment initiation for the five soil temperature fluctuation treatments (0, 4, 8, 12, and 16 °C)[†]. Treatment was used as an indicator variable (treatment of constant temperature was the reference group)

Fluctuation (Range of temperature)	**Parameter estimates**			
	Germinated sclerotia		*Apothecia*	
	Intercept	Days	Intercept	Days
0 °C (20-20 °C)	-11.77 [a]	0.45 [a]	-136.9 [a]	3.96 [a]
4 °C (18-22 °C)	-3.46 [e]	0.41 [a]	-97.7 [c]	4.60 [c]
8 °C (16-24 °C)	-25.29 [b]	1.23 [b]	-297.6 [b]	13.55 [b]
12 °C (14-26 °C)	-16.05 [c]	0.40 [a]	-136.3 [a]	3.89 [a]
16 °C (12-28 °C)	-4.55 [d]	0.24 [c]	-126.9 [a]	3.79 [a]
	($R^2 = 0.99$)		($R^2 = 0.98$)	

[a], [b], [c], [d], [e]: Parameter estimates in a column with the same letter did not different significantly at $P = 0.05$ (as determined with Student's *t*-test).

[†] : Temperature fluctuations of 0, 4, 8, 12, and 16 °C were generated with 20-20, 18-22, 16-24, 14-26, and 12-28 °C daily temperature fluctuations respectively with maximum temperature at noon and minimum at midnight.

Table 3.2. Parameter estimates of linear regression of total number of germinated sclerotia and apothecia of *Sclerotinia sclerotiorum* with number of days after experiment initiation for the four soil moisture fluctuation treatments (saturation, high, medium, and low soil water potential SWP)[†]. Treatment was used as an indicator variable (treatment of saturation was the reference group)

Fluctuation (Range of SWP)	**Parameter estimates**			
	Germinated sclerotia		*Apothecia*	
	Intercept	Days	Intercept	Days
Saturation (-0.001 bars)	-27.4 [a]	0.95 [a]	-226.4 [a]	6.51 [a]
High SWP (-0.3 to -0.4 bars)	-3.26 [b]	0.09 [b]	-13.47 [b]	0.27 [b]
Medium SWP (-0.6 to -0.7 bars)	-3.91 [b]	0.09 [b]	-10.36 [c]	0.26 [b]
Low SWP (-0.9 to -1.0)	-1.03 [c]	0.03 [c]	-2.11 [d]	0.06 [c]
	(R^2 = 0.97)		(R^2 = 0.94)	

[a], [b], [c], [d]: Parameter estimates in a column with the same letter did not different significantly at P = 0.05 (as determined with Student's t-test).

[†] : SWP was manipulated by drying the soil until ψ_m reached the values of -0.3 to -0.4 bars (high SWP), -0.6 to -0.7 bars (medium SWP), and -0.9 to -1.0 bars (low SWP). When ψ_m for a given fluctuation treatment had reached its final value, water was added to the crispers of the treatment until soil was saturated ($\psi_m \cong$ -0.001 bars).

Table 3.3. Frequency (%) of sclerotia of *Sclerotinia sclerotiorum* from the surface of which fungal species were isolated in five soil temperature fluctuation treatments (0, 4, 8, 12, and 16 °C) †

	Temperature fluctuation				
	0 °C	***4 °C***	***8 °C***	***12 °C***	***16 °C***
Fungi**	*Mean (range)*‡	*Mean (range)*	*Mean (range)*	*Mean (range)*	*Mean (range)*
Fusarium spp.	85.4 (81.2-89.6)^{b1*}	87.5 (83.3-91.7)^{b12}	95.8 (89.6-100)^{a2}	100 (100-100)^{a3}	100 (100-100)^{a3}
Trichoderma spp.	81.3 (81.2-83.3)^{b2}	83.3 (75-87.6)^{b12}	70.2 (23-89.5)^{b2}	-	-
Penicillium spp.	72.9 (71-79.1)^{c1}	70.8 (16.6-89.5)^{c1}	31.3 (8.3-64.8)^{c2}	-	-
Gliocladium spp.	31.3 (6.25-77.1)^{d1}	14.6 (2.1-35.45)^{d2}	6.3 (0-14.7)^{d3}	-	-
Coniothyrium spp.	18.8 (8.3-31.2)^{d1}	6.3 (0-14.6)^{d3}	4.2 (0.0-12.5)^{d3}	-	-
Alternaria spp.	10.4 (0.0-22.9)^{d3}	8.3 (0-18.6)^{d3}	-	-	-

* Values with the same letter-number did not differ significantly at $P = 0.05$ as determined with Student's *t*-test.

** At the end of each temperature experiment, sclerotia were plated in cornmeal agar (CMA) at 20 °C and a 12-h photoperiod for 7 to10 days. Fungi were further transferred to APDA and incubated for 7 to 10 days at 20 °C and a 12-h light dark cycle for identification.

† : Temperature fluctuations of 0, 4, 8, 12, and 16 °C were generated with 20-20, 18-22, 16-24, 14-26, and 12-28 °C daily temperature fluctuations respectively with maximum temperature at noon and minimum at midnight.

‡ : Temperature experiment was repeated 4 times. Numbers in parentheses are the lowest and highest frequencies for each fungal species recorded in the 4 experiments.

Table 3.4. Frequency (%) of sclerotia of *Sclerotinia sclerotiorum* from the surface of which fungal species were isolated in four soil water potential fluctuation treatments (saturation, low, medium, and high soil water potential) †.

	Moisture fluctuation			
	Saturation ‡	***High SWP*** ‡	***Medium SWP***	***Low SWP***
Fungi**	*Mean (range)*	*Mean (range)*	*Mean (range)*	*Mean (range)*
Fusarium spp.	86.6 (83-90)^{a1*}	91.6 (83-98)^{a2}	52.1 (42-63)^{a3}	26.4 (21-33)^{a4}
Trichoderma spp.	36.1 (21-48)^{b1}	39.6 (0-54)^{b1}	26.4 (19-40)^{b2}	9.7 (4-19)^{b3}
Penicillium spp.	83.3 (83-88)^{c1}	93.7 (92-96)^{c2}	98.6 (98-100)^{c3}	100 (100-100)^{c4}
Gliocladium spp.	14.6 (4-31)^{d1}	2.6 (0-8)^{d2}	11.1 (4-21)^{d1}	-

* Values with the same letter-number did not differ significantly at $P = 0.05$ as determined with Student's t-test.

** At the end of each moisture experiment, sclerotia were plated in cornmeal agar (CMA) at 20 °C and a 12-h photoperiod for 7 to 10 days. Fungi were further transferred to APDA and incubated for 7 to 10 days at 20 °C and a 12-h light dark cycle for identification.

† : Soil moisture was manipulated by drying the soil until ψ_m reached the values of -0.3 to -0.4 bars (high SWP), -0.6 to -0.7 bars (medium SWP), and -0.9 to -1.0 bars (low SWP). When ψ_m for a given fluctuation treatment had reached its final value, water was added to the crispers of the treatment until soil was saturated ($\psi_m \cong -0.001$ bars).

‡ : SWP: Soil Water Potential (ψ_m). SWP experiment was repeated 3 times. Numbers in parentheses are the lowest and highest frequencies recorded in the 3 experiments.

Table 3.5. Frequency (%) of sclerotia of *Sclerotinia sclerotiorum* from the surface of which, fungal species were isolated after 9 and 17 months in storage. Sclerotia were stored in room temperature until used in the experiments

	Frequency of fungal species **	
Fungi	***after 9 months in storage***	***after 17 months in storage***
Fusarium spp.	58	70
Alternaria spp.	12	16
Penicillium spp.	62	48
Gliocladium spp.	6	-

** Sclerotia were plated in cornmeal agar (CMA) at 20 °C and a 12-h photoperiod for 7 to 10 days. Fungi were further transferred to APDA and incubated for 7 to 10 days at 20 °C and a 12-h light dark cycle for identification.

CHAPTER IV. LOGISTIC REGRESSION MODELING OF PREVALENCE OF SOYBEAN SCLEROTINIA STEM ROT IN THE NORTH CENTRAL REGION OF THE UNITED STATES

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A. Abstract

Regional prevalence of soybean Sclerotinia stem rot (SSR), caused by *Sclerotinia sclerotiorum*, was modeled using tillage practices, soil texture, and weather variables from NOAA (monthly air temperature and monthly precipitation for the months of April to August) as input variables. Potential differences between states in disease prevalence were addressed using regional indicator variables. Logistic regression was used to estimate the probability of stem rot prevalence with historical disease data in four states in the North Central Region of the United States. Two models were selected, Model I uses spring weather conditions (April) and Model II uses summer weather conditions (July and August) as input variables. Both models had high explanatory power (78.5 and 77.8% for Models I and II, respectively). To investigate further the explanatory power of the models, each of the four states was divided into small geographic areas, and disease prevalence in each area was estimated using both Models I and II. The R^2 of the regression analysis between observed and estimated SSR prevalence were 0.65 and 0.71 for Model I and II, respectively. The same input variables were tested for their efficiency to explain the within field SSR incidence using Poisson regression analysis. Although all input variables were significant, only a small amount of the SSR incidence was explained. The R^2 of the regression analysis between observed and estimated SSR incidence was 0.065. Incorporation of available site-specific

information, i.e. fungicide seed treatment, weed cultivation, manure and fertilizer applications in a field, produced little improvement on the explained amount of SSR incidence. In this case, the R^2 of the regression analysis between observed and estimated SSR incidence was 0.076. Predicted field incidence was generally overestimated in both efforts comparing to the observed incidence. Our results suggest that preseason prediction of regional prevalence would be feasible. However, prediction of field incidence would not be, and a different site-specific approach should be followed.

B. Introduction

Soybean Sclerotinia stem rot (SSR), caused by *Sclerotinia sclerotiorum*, (Lib.) de Bary was first found in the United States in 1946 and reported in 1951 (7). Initially, the disease was a minor problem with localized epidemics occurring in areas of Michigan, Minnesota, and Wisconsin where soybeans of maturity groups 0 and I are grown (13). Since the beginning of the last decade, the disease distribution changed significantly, expanding to areas where maturity groups II and III are grown (25). Before 1991, in the North Central Region SSR was ranked twelfth in soybean disease losses (10), in 1994 it was second after soybean cyst nematode (27), while in 1998 it was ranked eleventh (28).

Previous research on the epidemiology of the pathogen indicates that cool and moist conditions during soybean flowering stages are favorable to disease development (2). SSR is found severe when temperature is low and moisture is high under the plant canopy (5). Soil texture seems to be important as well. It has been reported (19) that light-textured soils favored the germination and apothecial formation by sclerotia of *Sclerotinia sclerotiorum*.

Studies have shown that tillage affects SSR incidence (11,24). In soybeans, more apothecia were observed in minimum tillage than in no-tillage fields, although no significant differences in disease incidence among tillage treatments were found (11), while in another study in Alberta (24), plowing reduced sclerotial germination and apothecial production. Use of conservation tillage is often associated with narrow row soybeans which are more favorable to SSR than wide row soybeans due to the dense soybean canopy development (12,20).

Little is known on the regional distribution and prevalence of SSR. In a recent regional study (25) conducted in Illinois, Iowa, Minnesota, Missouri, and Ohio, the disease was found to be more prevalent when summer temperatures were below normal (relative to a 30-year average) than when they were above normal. Furthermore, the prevalence of the disease increased exponentially with latitude and was found further south in 1996, a year with a cooler-than-normal summer (25). In the same survey the prevalence of Sclerotinia stem rot was highest in minimum-till, second in conventional-till and lowest in no-till fields.

Few data are available on the study of regional plant disease prevalence. Most of the published studies focus on disease incidence, but there are no available comparative studies on disease prevalence and incidence. Such studies could help understand the relationship, if any, between two of the fundamental scales of plant diseases, regional disease prevalence and within-field incidence.

The primary objective of this study was to develop explanatory models of SSR prevalence in four states of the north-central region using tillage, soil texture, and climate variables as input variables. A secondary objective was to investigate if the within-field SSR incidence could be explained by the variables used to explain SSR regional prevalence. The variable selection was based on the biology and epidemiology of the pathogen (1,6,18,19) and on the preliminary results of our previous study (25).

C. Materials and methods

Data collection. Data collection has been previously described in detail (25, 26). In brief, soybean samples were collected in 1995 and 1996 from Illinois, Iowa, Minnesota, Missouri, and Ohio to determine the prevalence of Sclerotinia stem rot in collaboration with the National Agricultural Statistics Service (NASS). In 1997 and 1998, more samples were collected from Iowa, Minnesota, and Missouri. From each selected field, a liter or more of soil and 20 soybean stems were sampled in a zigzag pattern and shipped to Iowa State University for analyses. The zigzag pattern contained 10 corners, at which approximately 100 cm^3 of soil and 2 stems were collected. Each stem was externally observed for presence or absence of the typical cottony mycelium. The stems were also longitudinally split and checked for presence or absence of sclerotia in the pith. Isolation was made on acidified potato dextrose agar from stems with signs and symptoms that could not be readily identified. The soil collected from the 10 corners was bulked, thoroughly mixed, and approximately 1 liter was sub-sampled. The soil texture of each sample was determined by a commercial laboratory (MVTL Laboratories, Inc. Bismarck, ND) with methods previously described (26). Sclerotinia stem rot was not found in any sample from Missouri. Since the disease has not been reported in this state, data from Missouri were not included in model development. Overall, during the four years of investigation, 1,545 fields from Illinois, Iowa, Minnesota, and Ohio were sampled. Sclerotinia stem rot was detected in 87 fields. In the fields that SSR was detected disease incidence varied from 5 to 80%. In this study the term prevalence is used to describe the presence or absence of SSR in a soybean field, and the term incidence is used to describe the percentage of infected soybean plants in a field.

Tillage categories. Tillage systems were classified into three categories according to the amount of surface residue (3). Conservation tillage systems maintain greater than 30% surface residue after planting. Tillage practices that maintain 15 to 30% surface residues are categorized as minimum-till, whereas those that maintain less than 15% surface residue are classified as conventional till. For this research, tillage information for each field was obtained from farmers during interviews conducted by NASS.

Weather data. Weather data were obtained from the National Oceanic and Atmospheric Administration (NOAA) on-line through the National Climatic Data Center (NCDC) in Asheville, NC. Data included mean monthly air temperature and precipitation for the months of April, May, June, July, and August for the years 1995 to 1998 for Iowa and Minnesota and 1995 and 1996 for Illinois and Ohio.

In this study, mean monthly temperature and precipitation were obtained for each sampled field from the nearest weather station. The corresponding weather data were obtained from 101 weather stations in Illinois, 107 in Iowa, 51 in Minnesota, and 61 in Ohio. In 82% of the cases, the sampled fields were located less than 20 km away from the nearest weather station. Only in eighteen cases in Illinois, eight cases in Iowa, seven in Minnesota, and eleven cases in Ohio was the distance between field and weather station greater than 25 km.

Logistic regression analysis. Logistic regression was used to identify the factors significantly associated with regional prevalence of Sclerotinia stem rot. The dependent variable was absence or presence of the disease in a field; thus, fields were divided into two categories based on the presence or absence of the disease on the collected samples. Logistic regression is widely used in epidemiological research, where the binary response is usually the presence or absence of a disease and the predictor variables are putative risk factors and possible confounding variables (4,15). If Y represents disease presence in a field and only takes on values 0 and 1 (absence or presence of the disease) the probability of disease prevalence can be modeled as:

$$P(Y = 1) = \exp\{\ \Sigma b_i X_i\} / (1 + \exp\{\ \Sigma b_i X_i\}) \ . \qquad (1)$$

Here, b_i's are parameters to be estimated, and the X_i 's are the covariates or predictors. Because the response variable $P(Y = 1)$ is a probability, it is constrained to lie between 0 and 1; note that for any values of b_i and X_i, the logistic function given in equation (1) is also constrained to the interval [0, 1]. The parameters b_i are similar to the intercept and regression coefficients in an ordinary multiple regression model. However, their interpretations are somewhat different from these in logistic regression (15). They are used to quantify the *disease prevalence risk factor* of an area.

The LOGISTIC procedure in SAS (Statistical Analysis Systems: SAS Institute Inc., Cary, NC) was used to fit equation (1) to the data. To select the best set of predictors, the method of backward elimination was used. Starting from a saturated (or full) model, we sequentially dropped those covariates or predictors that did not appear to be significantly associated with the response variable ($P = 0.05$). In the final model, all predictors were significantly associated with disease prevalence.

Poisson regression analysis. Poisson regression is commonly used in the study of data taking the form of independent counts; in human epidemiology for instance the study of the incidence of non-contagious diseases typically uses the number of cases in an area as the response variable (4).

In case of SSR incidence the dependent variable was the number of diseased soybean plants in a field. If y represents number of diseased plants in a field and only takes values between 0 and 20 (20 is the maximum possible observation since 20 stems were collected from each field) the likelihood function to represent disease incidence is:

$$L(y) = \lambda^{y} e^{-\lambda} / y! \qquad (2)$$

with $\lambda = \exp(\Sigma b_i X_i)$ to ensure that y is positive since the Poisson distribution is defined for non negative integers.

Here, b_i's are parameters to be estimated, and the X_i 's are the covariates or predictors. The response variable $L(y)$ is a count, and in this case can take values 0,1,2,...20. The parameters b_i are used to quantify the *disease incidence risk factor* of a field. The GENMOD procedure in SAS (Statistical Analysis Systems: SAS Institute Inc., Cary, NC) was used to fit equation (2) to the data.

Criteria of goodness of fit. 1) *Logistic regression:* For all pairs of observations with different values of the response variable, a pair is called *concordant* when the observation with the larger ordered value of the response has a lower predicted event probability than the observation with the smaller ordered value of the response. A pair is called *discordant* when the observation with the larger ordered value of the response has a higher predicted event probability than the observation with the smaller ordered value of the response. A pair is called *tied* when it is neither concordant nor discordant. *Correlation indices*: Somers' D, Gamma, Tau-a, and c are computed from the numbers of concordant and discordant pairs of observations. A model with higher values for these indices has better predictive ability than a model with lower values for these indices. The best fitting models for prevalence were selected on the basis of the amount of variation explained by the predictors as measured by the criteria mentioned above.

2) *Poisson regression:* The deviance (equal to -2*log(Likelihood)) and the log(Likelihood) values were used to compare the two explanatory models tested for SSR incidence. Models with good fit have low deviance and high log(Likelihood) values. The difference in deviance was used to compare the two fitted incidence models. The significance of the difference in deviance was evaluated using a chi-square test with the appropriate degrees of freedom (8).

Input variables. *Prevalence.* Input variables were: monthly average air temperature and total precipitation for the months of March to August, soil texture, percent of sand, percent of clay, and percent of silt in the soil. Also two indicator variables were used. One was used to capture tillage effect, and the other was used to capture any regional effect. In the first case, conventional tillage was selected as the reference group, so that the effect of other types of tillage was estimated relative to the effect of the conventional tillage. In the case of region, the state of Illinois was used as the reference group and the SSR prevalence risk in Iowa, Minnesota, and Ohio was estimated relative to the risk in Illinois.

Variables were tested for possible correlation (using the CORR procedure of the SAS software) since the presence of collinearity among predictors in a model reduces the accuracy with which parameters can be estimated. There was high correlation between percent of sand and percent of silt, percent of sand and percent of clay. Also the high positive correlation between spring and summer weather variables (Table 4.1) suggests that incorporation of all weather variables into the model would lead to inefficient estimates of the regression coefficients. The long-term weather pattern of the area does not support the assumption that the correlations found during the four-year period of investigation represent the general weather pattern of the north central region. The average air temperatures of April, May, June, and the average of July and August from 1961 to 1999 show that in Iowa low spring temperature can be followed by either high or low summer temperature (Fig. 4.1). For the reason, spring and summer variables were not used simultaneously, and two categories of models were developed separately: one using spring weather variables (candidate Models I) and one using summer weather variables (candidate Models II). Similarly, only clay was used for describing soil texture.

Incidence. Input summer weather variables used for modeling SSR prevalence were tested also as candidate variables for explaining the within soybean fields SSR incidence (5,12,19). Furthermore, several other management practices that had been applied in the sampled fields were incorporated for modeling SSR incidence. These management practices were: fungicide seed treatment, manure and fertilizer applications, and weed cultivation. All of them were used as indicator variables with no application of a management practice used as the reference group. The issue of collinearity between management practices and management practices and weather variables has been previously addressed (14). Most of the input variables used for SSR incidence were highly correlated. Thus, we should note that parameter estimates may not be efficient.

Presentation of estimates and explanatory power of models. The four states in the North Central Region were arbitrarily divided into smaller geographic areas. For each of these areas, the estimated disease prevalence for each year from 1995 to 1998 was calculated using Model I (April weather variables) and Model II (July and August weather variables). The PROB procedure of SAS was used to estimate SSR prevalence and incidence for each sampled field in the area. Estimated values were then averaged to produce the estimated SSR prevalence for the area. Estimated prevalence for each area was plotted against observed prevalence, and regression analysis was performed between the estimated and observed values. Similarly, estimated incidences calculated with PROB procedure of SAS were regressed against observed incidences in sampled fields so that estimation accuracy could be investigated.

D. Results

Correlation between weather variables. High correlations were found between April temperature and April precipitation, April temperature and May temperature, April temperature and May precipitation, and April temperature and average temperature of July and August (Table 4.1). Significant correlations were also found between April precipitation and average temperature of July and August, May temperature and average temperature of July and August, May precipitation and average temperature of July and August, and June temperature and average temperature of July and August.

Logistic regression results for SSR prevalence. The two best fitting models were: a) Model I where April precipitation, April air temperature and its interactions with the indicator variables for tillage and regional effects were the input variables (Table 4.2), and b) Model II where July precipitation, average air temperature of July and August as well as the interaction between average temperature of July and August and tillage and state indicator variables were the input variables (Table 4.3). For both models goodness of fit was quantified by the proportion of concordant and discordant pairs, the values of Somers' D, Gamma, Tau-a, and c statistics (Table 4.4). Clay and August precipitation were not significant explanatory variables ($P = 0.05$).

Observed and estimated prevalence of SSR using Model II for Illinois and Ohio in 1995 and for Iowa and Minnesota in 1998 are given in Fig. 4.2. Both models (I and II) gave good estimation for all the areas of Illinois, Iowa, Ohio, and the three lower areas of Minnesota. In the three upper areas of Minnesota, the estimated prevalence was higher than the observed prevalence in 1995, 1996, and 1998 and lower in 1997.

The comparison between observed and estimated SSR prevalence for each geographic area given by Model I and II respectively is presented in Figs. 4.3 and 4.4. There is a large number of areas where SSR observed prevalence was very low and only two areas with high SSR prevalence (Fig. 4.3A). Both Models I and II redistribute the very low prevalence values in the low classes of estimation (between 0 and 0.1) and underestimate the very high prevalence values (Figs. 4.3B and C), smoothing the SSR regional picture as commonly happens when large-scale modeling is attempted. In 89% of the cases in which the actual prevalence was zero, both Models I and II gave lower than 0.1 prevalence. In the rest of the cases (11%), the estimated prevalence was between 0.1 and 0.14. The R^2 values between the observed and estimated values of prevalence were 0.65 for Model I and 0.71 for Model II (Fig. 4.4). The intercept of the regression between estimated and observed values was not significantly different from zero ($P > 0.05$) and the slope was not significantly different from 1 for both Model I and Model II.

Poisson regression results for SSR incidence. Summer weather variables, state and tillage indicator variables used for modeling SSR prevalence were also found significant for the SSR field incidence (Table 4.5). Interestingly, parameter estimates from Poisson regression for the SSR incidence were similar to the estimated parameters from logistic regression accounting for the SSR prevalence (Table 4.3). The main difference was that clay and August precipitation were significant for the SSR incidence while these variables were not found significant for the SSR prevalence. Actually, the August precipitation parameter estimate indicates that its effect is equivalent to the July precipitation effect (Table 4.5). When estimated incidences were regressed against observed values the R^2 was very low (0.065). The intercept of the regression between estimated and observed values was significantly different from zero ($P > 0.05$) and the slope was not significantly different from 1.

In addition, we incorporated in the Poisson regression analysis more information on management practices available from the sampled fields. The results of Table 4.5 show that all the newly incorporated management practices were significant for the SSR field incidence. All of them (manure, fertilizer application, seed treatment, and weed cultivation) seem to have higher effect on SSR incidence than the weather input variables. Furthermore, the difference in deviance between the two models (Table 4.5) suggests that the new analysis accounts significantly for more SSR incidence variability than the first one. When estimated incidences were regressed against observed values the R^2 was again very low (0.076). The intercept of the regression between estimated and observed values was significantly different from zero ($P > 0.05$) and the slope was not significantly different from 1.

E. Discussion

Non linear probability models, logistic and Poisson regression analyses were used to quantify two of the principal components of plant epidemiology, disease prevalence and disease incidence. Air temperature, precipitation, and tillage practices account significantly for the variation in the prevalence of Sclerotinia stem rot in four states of the north central region, and allow for the development of models with high explanatory power. When the same variables were tested with Poisson regression only a small portion of SSR field incidence could be explained. Our results suggest that preseason prediction of regional prevalence would be feasible. However, prediction of field incidence would rather need a different site-specific approach.

Two models were selected for explaining SSR prevalence. Model I uses April weather conditions and Model II uses July and August weather conditions as input variables. Correlation between observed and estimated disease prevalence was highly significant; the coefficients of determination from the regression of observed and estimated prevalence were 0.65 and 0.71 for Model I and II, respectively.

The selection of weather variables was based on results of previous studies (1,2,19). Workneh and Yang (25) reported the significance of July and August air temperature to Sclerotinia stem rot spatial distribution. No reports were found on April weather to explain prevalence of Sclerotinia stem rot. However, April weather data were used in our model development to investigate their possible significance on explaining SSR prevalence. It would be useful to have pre-season indicators of the expected SSR prevalence during the upcoming growing season.

We used four years of data from all states (Iowa, Illinois, Ohio, and Minnesota) to increase the inter-year weather variation. However, the high correlation between monthly weather variables suggests that four years of data may be too short of a sequence with regards to weather variation (R. Carlson, *personal communication*). Due to the high correlation between some input variables (Table 4.1), different combinations of covariates were tested in model selection. Use of air temperature and precipitation of April resulted in a model with high explanatory power ($R^2 = 0.79$). However, because of the high correlation between April precipitation and July and August air temperatures, there might be confounding between these effects. That is, the importance of April precipitation on Sclerotinia stem rot prevalence may be due to the correlation of this variable with air temperature of July and August, factors that were found to be significant to the development of SSR epidemics (25).

The significance of July and August average temperature agrees with previous investigations (6,18,23,25) on disease development based on the pathogen biology and epidemiology. July and August air temperatures were used as one variable (average) since the high positive correlation between them does not allow separating these two months as two independent predictors. Workneh and Yang (25) reported that precipitation was not a limiting factor for SSR prevalence in the north central region in 1995 to 1998. The conclusion was made examining 10 locations (5 in Iowa and 5 in Minnesota). In the present study, we considered 1,545 locations (fields) located in a large region that stretched from the south of Illinois to the north of Minnesota. The finding of significance of precipitation on prevalence as a predictor may be due to the large number of locations distributed in a broader geographic area used in the present study.

In our analysis, minimum tillage contributed significantly to disease prevalence in an area, while no significant difference was found between conventional tillage and no-tillage. This result is not in complete agreement with previous analysis (25) where prevalence of Sclerotinia stem rot was reported to be significantly less in no-till fields than in conventional or minimum-till fields and greater in minimum-till than in conventional-till fields. In that case, tillage was examined independently of any other factors, while in the present study more factors were incorporated into the model.

Though both Model I and Model II incorporate weather variables from different months, they present a similar structure: in both models air temperature and precipitation are significant with the significance of air temperature depending upon tillage practices and geographic position. Both models predict a higher risk for Iowa and Minnesota and reveal the importance of minimum tillage in the regional prevalence of Sclerotinia stem rot.

Both models tend to overestimate SSR prevalence when the observed prevalence is low. This overestimation was mainly observed in the upper three areas of Minnesota where the number of sampled fields was small all the years of the investigation and thus may not be sufficiently informative about actual levels of disease prevalence in those areas. For instance, there was only one sampled field for 1998 from the areas in Minnesota with predicted prevalence 0.11 (Fig. 4.2). Thus, the observed prevalence could be only 0 or 100%, and in no case could be in close accordance with our predictions.

Based on previous investigations (6,12,22,25) and given the limitations of the available data, we argue that Model II, which includes average air temperature of July and August, precipitation of July, and tillage practices, provides more reliable estimates of the regional prevalence of Sclerotinia stem rot in the north central region than does Model I.

SSR incidence could not be adequately explained by the same variables used to model SSR prevalence. Use of regional weather variables as inputs for incidence prediction model generally overestimated SSR incidence, indicating that site-specific environment conditions are less inducive to SSR compared to regional weather condition. It is well established in the literature that SSR incidence is affected by a number of microclimatic factors (1,17,21,22). Environmental factors like daily air temperature under the soybean canopy, frequency and total daily rainfall or soil moisture are more appropriate variables to describe SSR incidence than monthly average temperature and total precipitation (6,18,19,21,23). Also management practices that were not considered in the study such as soybean varieties, and row spacing may account significantly for the SSR field incidence (12,20,29). Furthermore, information on the field inoculum level is necessary, though the relationship between inoculum level and SSR incidence is not well defined (9,14,16).

It is generally true that decision on pest management is based on field-level information. Farmers will make their decision on pest management based on their field characteristics and anticipated level of disease so that they minimize their expected losses. In the case of SSR that means that farmers will have to incorporate information on the effect of management practices on SSR incidence. However, it has been demonstrated that most of the management practices have a complicated relationship with soybean attainable yield too, which adds uncertainty to the farmers' decision process (17). Further, farmers would need to collect information on daily temperature and precipitation under the soybean canopy so that the anticipated level of SSR incidence can be defined with a high degree of accuracy.

SSR prevalence was high in the north central region in 1996 to 1998. After that, the disease pressure in the states used in the present study (Illinois, Iowa, Minnesota, and Ohio) diminished significantly and currently SSR prevalence is very low even in Minnesota (J. Kurle, *personal communication*). Under these circumstances, it is rather questionable whether farmers will be willing to spend time and effort collecting field information to determine the anticipated SSR incidence, or change their management practices to minimize a low and rare disease risk. In the current literature (17,30), it has been suggested that for rare diseases farmers may depend mainly on their past experience to make decisions. If this is the case, prevalence could be practically effective information for decision making though less precise and informative than incidence.

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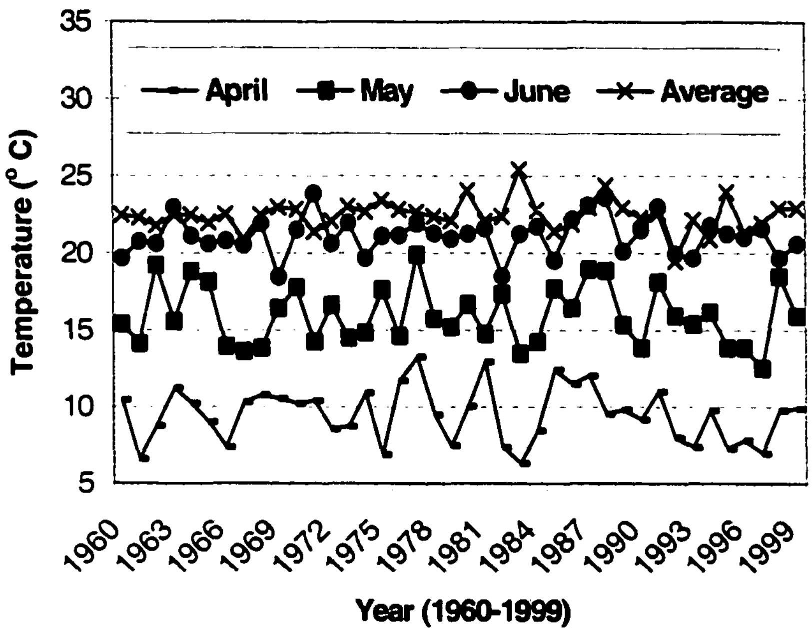

Fig. 4.1. Monthly air temperature of April, May, June, and average of July and August for the years between 1960 and 1999 in Iowa.

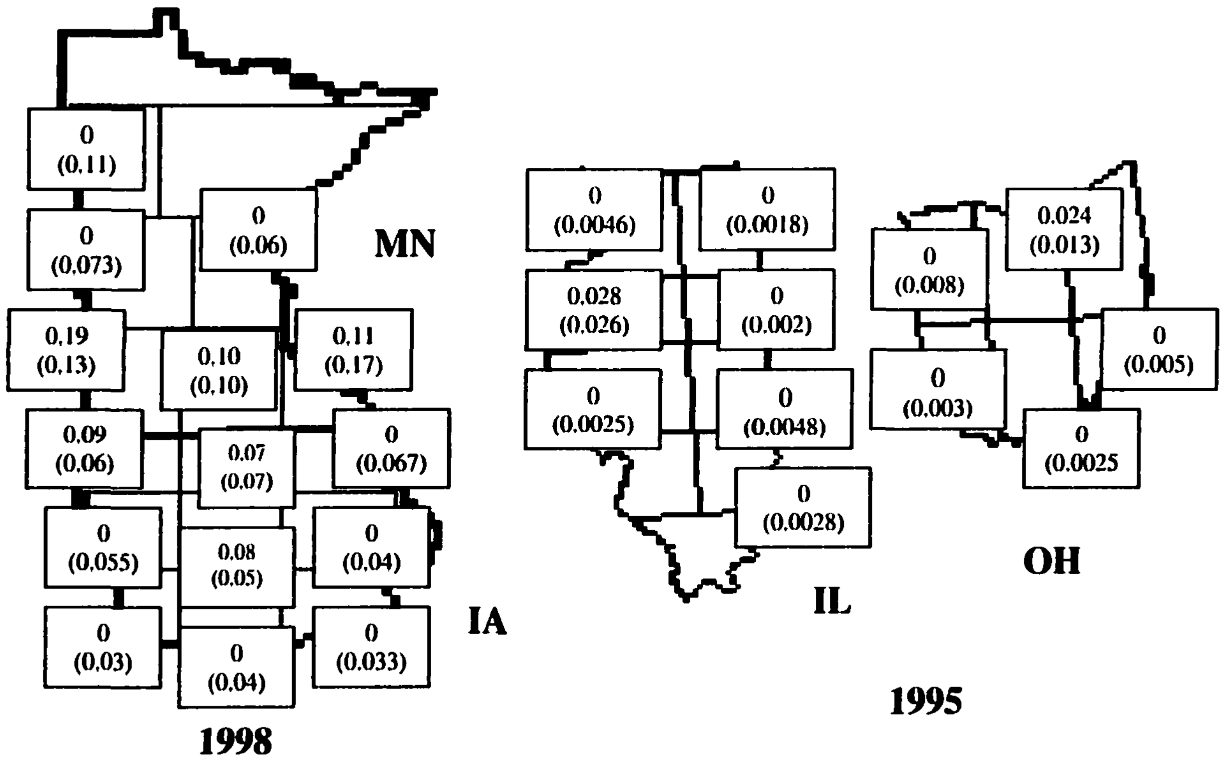

Fig. 4.2. Observed frequency and estimated prevalence (parenthesis) of soybean Sclerotinia stem rot (caused by *Sclerotinia sclerotiorum*) in Illinois (IL) and Ohio (OH) in 1995, and in Iowa (IA) and Minnesota (MN) in 1998 using tillage and July and August weather as input variables (Model II).

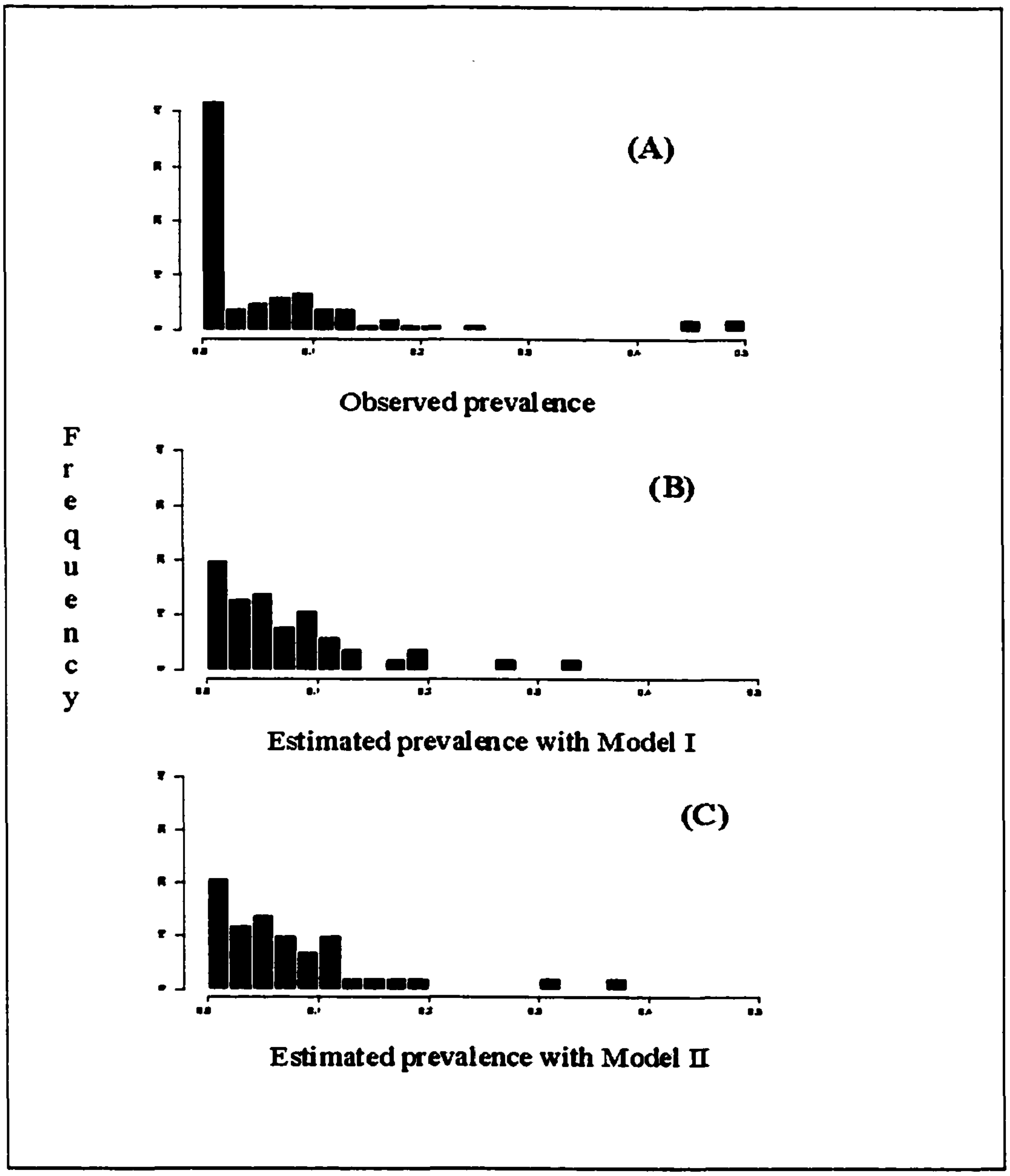

Fig. 4.3. Frequency of observed (A), estimated with Model I (B), and estimated with Model II (C) prevalence of Sclerotinia stem rot (*Sclerotinia sclerotiorum*) during the four year survey (1995-1998) in the sub-areas of Fig. 4.2.

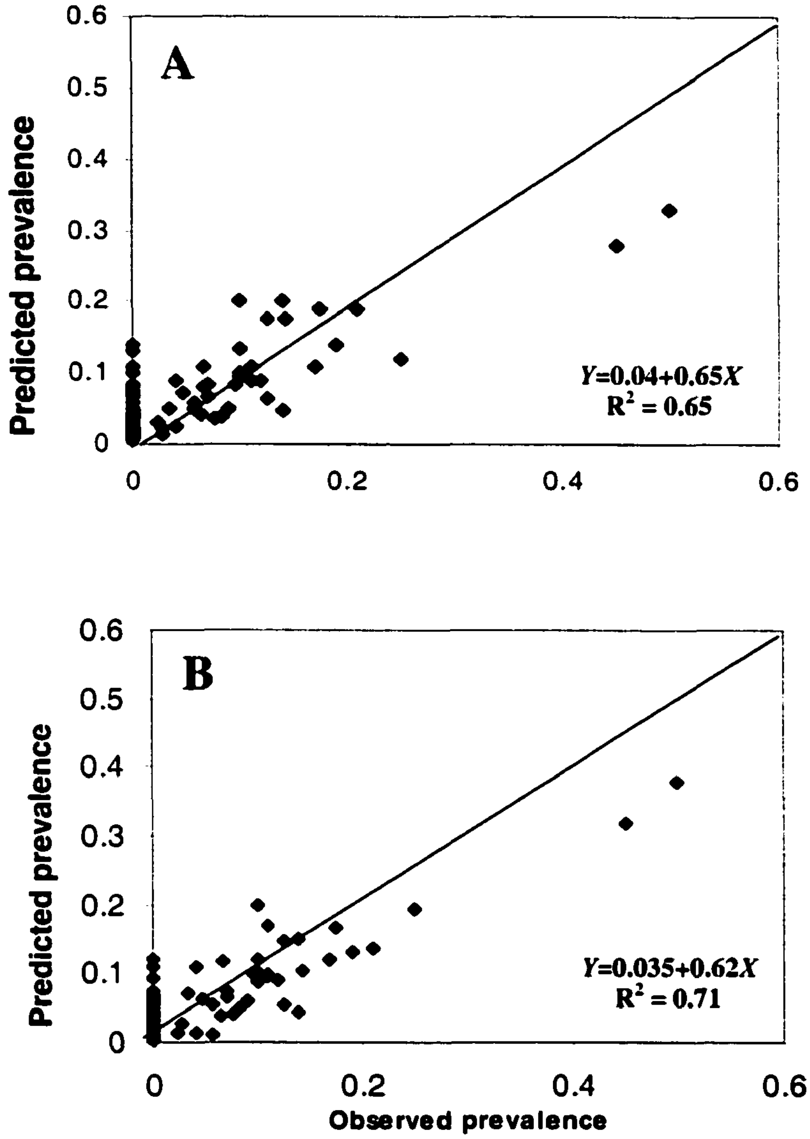

Fig. 4.4. Comparison of the predicted and the observed prevalence of soybean Sclerotinia stem rot (*Sclerotinia sclerotiorum*) during the four year survey (1995-1998) for **(A)** Model (April weather variables) and **(B)** Model II (July and August weather variables). If a predicted value is equal to the observed value this point should be on the line. Model parameter estimates are in Tables 4.2 and 4.3.

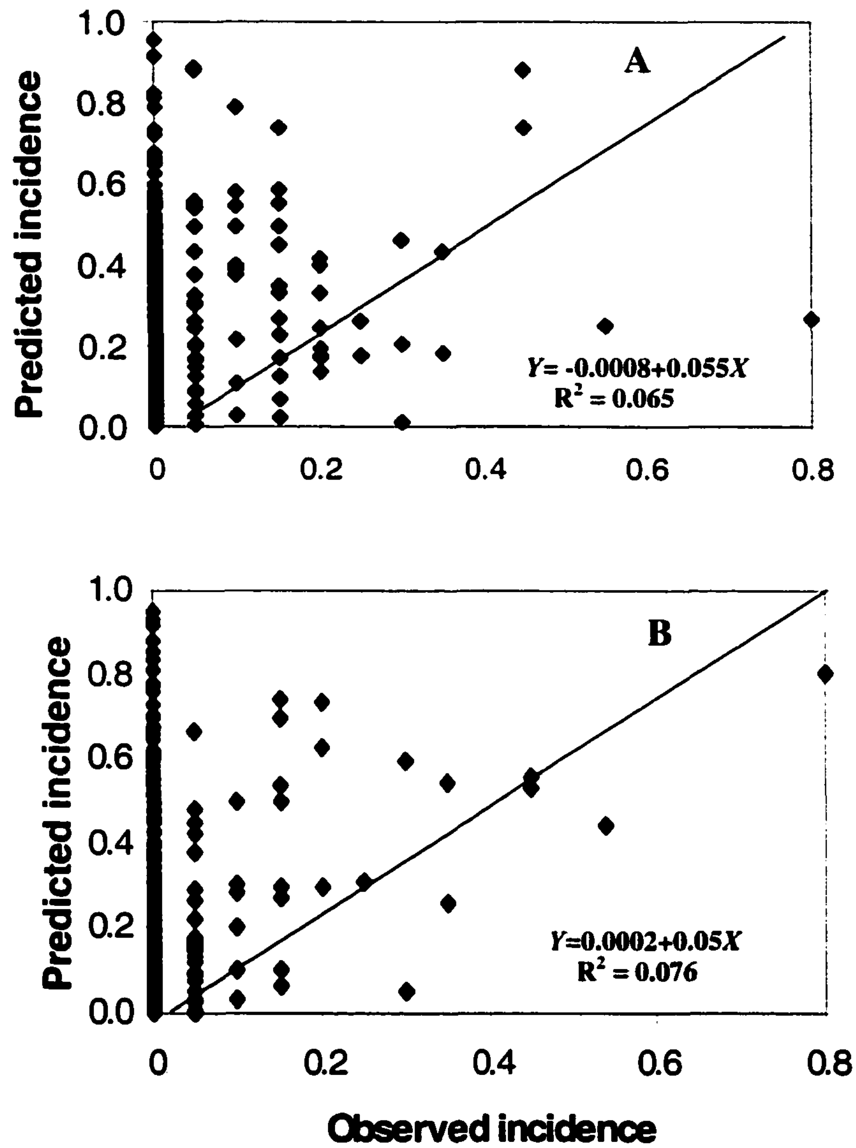

Fig. 4.5. Comparison of the predicted and the observed incidence of soybean Sclerotinia stem rot (*Sclerotinia sclerotiorum*) during the four year survey (1995-1998) when **(A)** tillage and summer weather, and **(B)** summer weather and management practices are used as model input variables. If a predicted value is equal to the observed value this point should be on the line. Model parameter estimates are in Table 4.5.

Table 4.1. Correlation coefficients among mean monthly temperature of April (APRT), May (MAYT), June (JUNT), average of July and August (AVERAGE) and April (APRPR), May (MAYPR), June (JUNPR), July (JULPR), August (AUGPR) precipitation in the north central region of the USA during 1995 to 1998

	APRT	APRPR	MAYT	MAYPR	JUNT	JUNPR	AVERAGE	JULPR	AUGPR
APRT	1.00[a]	**0.39***	**0.83***	**0.50***	**0.37***	0.21	**0.63***	-0.07	-0.07
	0.0[b]	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0029	0.0033
	1626[c]	1611	1626	1611	1626	1616	1609	1616	1616
APRPR		1.00	**0.43***	0.22	0.42	-0.01	**0.55***	0.01	-0.21
		0.0	0.0001	0.0001	0.0001	0.6546	0.0001	0.7145	0.0001
		1644	1611	1644	1611	1639	1606	1639	1639
MAYT			1.00	0.26	0.14	0.28	**0.50***	-0.06	-0.04
			0.0	0.0001	0.0001	0.0001	0.0001	0.0087	0.116
			1626	1611	1626	1616	1609	1616	1616
MAYPR				1.00	**0.44***	-0.06	**0.44***	0.02	-0.09
				0.0	0.0001	0.0098	0.0001	0.4011	0.0002
				1644	1611	1639	1606	1639	1639
JUNT					1.00	-0.25	**0.57***	-0.01	-0.13
					0.0	0.0001	0.0001	0.7300	0.0001
					1626	1616	1609	1616	1616
JUNPR						1.00	-0.09	0.02	0.14
						0.0	0.0001	0.3404	0.0001
						1648	1611	1648	1648
AVERAGE							1.00	-0.14	0.006
							0.0	0.0001	0.8048
							1622	1611	1611
JULPR								1.00	-0.17
								0.0	0.0001
								1648	1648
AUGPR									1.00
									0.0
									1648

[a] Pearson correlation coefficient.
[b] *p*-value for a significance test.
[c] number of observations.

* Bold values represent high correlation coefficients.

Table 4.2. Parameter estimates of the logistic regression used to explain the prevalence of soybean Sclerotinia stem rot (*Sclerotinia sclerotiorum*) using spring weather variables and tillage practices (Model I) in Iowa, Illinois, Minnesota, and Ohio for the years 1995 through 1998. Variables are significant different from 0 ($P = 0.05$)

	Region	**Variable**	**Parameter Estimate**	**Standard Error**
Conventional or No-till	Illinois and Ohio	Intercept	-1.874	1.37
		AprilT [a]	-0.162	0.006
		AprilPr [b]	-0.012	0.00035
	Iowa	Intercept	-1.33	1.37
		AprilT	-0.13	0.006
		AprilPr	-0.012	0.00035
	Minnesota	Intercept	-0.93	1.37
		AprilT	-0.11	0.0063
		AprilPr	-0.012	0.00035
Min-till	Illinois and Ohio	Intercept	-1.4	1.37
		AprilT	-0.135	0.0059
		AprilPr	-0.012	0.00035
	Iowa	Intercept	-0.85	1.37
		AprilT	-0.164	0.0059
		AprilPr	-0.012	0.00035
	Minnesota	Intercept	-0.46	1.37
		AprilT	-0.083	0.0063
		AprilPr	-0.012	0.00035

[a] AprilT: Temperature of April (° C), [b] AprilPr: Precipitation of April (cm)

Table 4.3. Parameter estimates of the logistic regression used to explain the prevalence of soybean Sclerotinia stem rot (*Sclerotinia sclerotiorum*) using summer weather variables and tillage practices (Model II) in Iowa, Illinois, Minnesota, and Ohio for the years 1995 through 1998. Variables are significant different from 0 ($P = 0.05$)

	Region	**Variable**	**Parameter Estimate**	**Standard Error**
Conventional or No-till	Illinois and Ohio	Intercept	5.708	3.66
		Average [a]	-0.46	0.0095
		JulyPr [b]	-0.029	0.0002
	Iowa	Intercept	6.22	3.66
		Average	-0.432	0.0096
		JulyPr	-0.029	0.0002
	Minnesota	Intercept	6.44	3.66
		Average	-0.42	0.0096
		JulyPr	-0.029	0.0002
Min-till	Illinois and Ohio	Intercept	6.06	3.66
		Average	-0.441	0.0096
		JulyPr	-0.029	0.0002
	Iowa	Intercept	6.572	3.66
		Average	-0.412	0.0096
		JulyPr	-0.029	0.0002
	Minnesota	Intercept	6.8	3.66
		Average	-0.40	0.0096
		JulyPr	-0.029	0.0002

[a] Average: Mean average temperature of July and August (^{o} C), [b] JulyPr: Precipitation of July (cm)

Table 4.4. Criteria of goodness of fit of soybean Sclerotinia stem rot (*Sclerotinia sclerotiorum*) prevalence models (Model I and Model II) for the four states of the north central region of the USA

Measures	Model I	Model II
Concordant (%) [a]	78.1	77.4
Discordant (%) [a]	21.1	21.7
Tied (%) [a]	0.8	1.0
Somers' D [b]	0.571	0.557
Gamma [b]	0.575	0.562
Tau-a [b]	0.061	0.057
c [b]	0.785	0.778

[a] measurements assess the association of estimated probabilities and observed responses.
[b] indices computed from the two first measurements. A model with higher values for these indices has better predictive ability than a model with lower values.

Table 4.5. Parameter estimates of the poisson regression used to explain the incidence of soybean Sclerotinia stem rot (*Sclerotinia sclerotiorum*) using summer weather variables and management practices

Parameter	*Estimate*	*Standard error*	*Estimate*	*Standard error*
Intercept	6.88*	1.3	13.28*	2.08
Average temperature of July and August	-0.46*	0.055	-0.68*	0.09
IA	0.71*	0.28	0.48	0.35
MN	0.98*	0.29	0.46	0.37
OH	-1.67**	0.75	-2.1*	0.77
July precipitation	0.028*	0.01	0.033***	0.02
August precipitation	0.037*	0.014	0.021	0.02
(Average temperature of July and August)x tillage)	-0.022***	0.013	-0.027***	0.016
(Average temperature of July and August)x(tillage)	0.024*	0.006	0.008	0.01
Clay			-0.03**	0.016
June precipitation			0.044***	0.024
Seed treatment			1.63***	0.87
Manure			-18.63*	5.44
Weed cultivation			2.42**	1.14
Fertilizer application			-16.59*	3.63
(Seed treatment)x(August prec)			-0.24**	0.11
(Manure)x(Average temperature)			0.83*	0.23
(Weed cultivation)x(June prec)			-0.37*	0.14
(Fertilizer)x(clay)			0.63*	0.15
(Fertilizer)x(Average temperature)			0.082*	0.03
Deviance	***1195***		***628***	
Log(Likelihood)	***-528.7***		***-286.8***	

Significant at: * 1%, ** 5%, and ***10% levels.

CHAPTER V. BAYESIAN LOGISTIC REGRESSION OF SOYBEAN SCLEROTINIA STEM ROT PREVALENCE IN THE U.S. NORTH-CENTRAL REGION: ACCOUNTING FOR UNCERTAINTY IN PARAMETER ESTIMATION

An invited symposium paper submitted to Phytopathology

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A. Abstract

Bayesian ideas have recently gained considerable ground in several scientific fields mainly due to the rapid progress in computing resources. Nevertheless, in plant epidemiology Bayesian methodology is not yet commonly discussed or applied. Results of a logistic regression analysis of a four-year data set collected between 1995 and 1998 on soybean Sclerotinia stem rot (SSR) prevalence in the North-Central Region of the United States were re-examined with Bayesian methodology. The objective of this study was to explore the level of uncertainty associated with the parameter estimates derived from the logistic regression analysis of SSR prevalence using Bayesian methodology. Our results suggest that the four-year data set used in the logistic regression analysis of SSR prevalence in the north-central region of US may not be informative enough to produce reliable estimates of the effect of some explanatory variables on SSR prevalence. Such confident estimations are necessary for deriving robust conclusions and high quality predictions.

B. Introduction

Plant pathologists face a variety of challenges every time they analyze their experimental results, fit probability models in complex data sets, draw conclusions about the present and make predictions for the future. Currently, most statistical analyses are performed with the help of commercial software packages, most of which use methods based on the framework of classical statistical philosophy. In this framework, maximum likelihood estimates (MLEs) and hypothesis tests based on p-values figure prominent.

The idea behind maximum likelihood parameter estimation is to determine the parameter values that maximize the probability (likelihood) of the sample data, i.e. values that are most consistent with the sample data. In general, the sample size is associated with the accuracy of an estimator. If the sample equals the entire population, then the estimator is the true value. From a statistical point of view, the method of maximum likelihood yields estimators with good statistical properties. Uncertainty associated with parameter estimation is quantified through confidence bounds that are often established using large sample (asymptotic) arguments.

Bayesian parameter estimation methods, in contrast, rely not only on current knowledge (sample data) but also on prior information that may be available on the parameters of interest. Thus, an important difference between the classical and the Bayesian framework is the introduction of prior information in the form of probability distributions. Moreover, in the Bayesian framework conclusions about parameters are made in terms of a *probability* statement, i.e. parameter estimates are no longer expressed as point estimates but instead are statistical distributions. Uncertainty associated with parameter estimation is quantified through the use of these probability distributions (7).

In a previous study (12), regional prevalence of soybean Sclerotinia stem rot (SSR), caused by *Sclerotinia sclerotiorum*, was analyzed using a logistic regression model. Estimation of the probability of stem rot prevalence was made with disease data from four states (Illinois, Iowa, Minnesota, and Ohio) in the North Central region of the United States.

Tillage practices, average air temperature of July and August, and precipitation in July and August were used as input variables. Potential differences between states in SSR prevalence were addressed by including regional indicator variables in the regression model. Because the parameter estimates were made with the classical approach, it would be interesting to examine the level of uncertainties associated with these estimates using Bayesian methodology.

Although scientists do not typically focus on assessing uncertainties associated with parameter estimation or model prediction, these are always present. Thus, one challenge is to accurately identify those variables in the model for which the sample data do not provide enough information for a reliable estimation and to investigate the possibility of improving estimation through the incorporation of any available prior information. Objective of the present study was to evaluate the level of uncertainty associated with the parameter estimates derived from the logistic regression analysis of SSR prevalence in the North-Central Region of the United States using data collected between 1995 and 1998 (12). We aimed at investigating if the four-year data set was informative enough so that the derived estimates for the explanatory variables of SSR prevalence were reliable.

C. Materials and methods

Concepts of Bayesian analysis. In the Bayesian framework, there are three key components associated with parameter estimation: the prior distribution, the likelihood function and the posterior distribution. These three components are formally combined in Bayes' rule:

$$\underbrace{f(\theta \mid y)}_{\text{posterior}} \propto \underbrace{g(\theta)}_{\text{prior}} * \underbrace{L(y \mid \theta)}_{\text{likelihood}} \qquad (1)$$

distribution distribution function

where the symbol $\propto$ denotes proportionality (7). In simple terms, equation (1) states that the information contained in the sample (reflected in the likelihood function) is combined with information from other sources (summarized by the prior distribution). The posterior distribution contains all the available knowledge about the parameters in the model.

Existing evidence about the parameters of interest may be available from earlier studies or from experts' opinions, and can be formalized into what is called the *prior distribution* of the parameter of interest (Fig. 5.1). A prior distribution can be non informative, informative or very informative. Non informative prior distributions are used in cases where no extra-sample information is available on the value of the parameters of interest. In statistical terms, this lack of knowledge is represented with a distribution that attributes approximately the same probability to each possible parameter value (Fig.5.1A). Informative prior distributions are used when some prior knowledge about the parameters of interest is available, such as when existing belief or evidence indicates that a parameter should get a value within a range (6). Formally, this knowledge is represented with a distribution that, for example, has a known mean and large variance (Fig. 5.1B). Very informative prior distributions are used when very strong prior knowledge about the parameters of interest is available, such as when existing belief or evidence indicates that a parameter of interest should get a specific value. In statistical terms this knowledge is represented with a distribution that has a known mean and small variance (Fig. 5.1C). The choice of an informative prior distribution typically involves a certain amount of subjectivity; historically, this has been a reason for disagreement between Bayesian and classical statisticians.

Currently collected data are used to form the *likelihood function.* The relative contributions of the likelihood function and the prior distribution to the posterior depend on the sample size; when the sample size is very large it is said that the data *dominate* the prior information i.e. the parameter estimates are based mainly on the information residing in the sample. It is important to notice that the likelihood function used in Bayesian analysis also forms the basis of classical statistical analysis. Thus, the main differences between classical and Bayesian modeling are the introduction of the prior distributions and the interpretation of the results.

SSR prevalence study. *Likelihood function.* In our SSR prevalence study, the likelihood function is based on the data set obtained through a survey conducted from 1995 to 1998 in Illinois, Iowa, Minnesota, and Ohio. In total, 1853 fields were randomly sampled during the four-year period. From each selected field, 20 soybean stems were sampled in a zigzag pattern and shipped to Iowa State University for examination. Survey methods have been previously described (16). *Prior distributions.* For comparison, two sets of prior distributions for the model parameters were examined: non informative, and very informative. The hierarchical model structure is explained in the appendix.

Explanatory variables. Explanatory variables used in the present study were selected based on a previous logistic regression analysis (12). These were: average air temperature of July and August, total precipitation of July and August, an indicator variable for tillage effect (with conventional tillage arbitrarily used as the reference group), and an indicator variable to represent state effect (with Illinois arbitrarily used as the reference group).

Assumptions used to construct very informative prior distributions. The following assumptions were made for defining the very informative prior parameter distributions: 1)

The regression coefficient associated with average air temperature of July and August should be negative. This assumption is based on previous literature (1,3,8) that Sclerotinia stem rot is a cool temperature disease, 2) The regression coefficient associated with July and August precipitation should be positive. Several epidemiological studies (1,3,13,15) demonstrate the importance of precipitation for disease occurrence since prolonged periods of high soil moisture are favorable for apothecial development (2). 3) Previous studies (1,3,13,14) show that precipitation around flowering is a main limiting factor for the disease development. Given this epidemiological aspect of Sclerotinia diseases that seems to be similar in more than one crop it was remarkable that the regression parameter estimate from logistic regression associated to July precipitation was one tenth of the parameter estimate for the average temperature of July and August. Further, it was rather unexpected to obtain an August precipitation parameter estimate that was not significant at the 5% level of significance (Table 5.1). The above estimations indicate that temperature and not precipitation is the most important factor for soybean SSR prevalence in the north-central region of US. However, since only four years of data were used in the logistic regression analysis it is questionable whether these data provide a reliable estimate of the effect of precipitation on the SSR prevalence. To address this question very informative prior distributions were imposed on July and August precipitation effect: we assumed that July precipitation effect is equal to the temperature effect (i.e. mean of prior distribution equal to 0.4), and August precipitation effect is half of July precipitation effect (i.e. mean of prior distribution equal to 0.2), 4) Based on previous studies (5,10) minimum tillage was the system with the highest risk and no tillage the system with the lowest risk, and 5) From the 4 states used in the analysis Minnesota was considered to have the highest risk and Ohio the lowest risk. To form the very informative prior distribution for the state effect we used a technique called Empirical Bayes, i.e. we estimated our prior parameters using information from the sample. The reason for using this technique was that there was no previous survey on SSR in the North-Central region to provide prior information on the relative risk of the disease in each state.

It should be clarified that the use of informative prior distributions was primarily to examine the degree of uncertainty associated with the parameter estimates. If the purpose is to improve the parameter estimations, detailed and precise information on the effect of each factor on SSR prevalence is needed.

Statistical analysis. Estimation of the posterior distributions of the parameters was carried out using the Gibbs sampler (GS). GS is an iterative algorithm based on a Markov chain theory that permits empirical estimation of posterior distributions. The analysis was implemented with BUGS (Bayesian Using Gibbs Sampling) software (WinBugs, version 1.3). CODA, a suite of S-plus (Mathsoft, Inc) functions, was used for plotting the BUGS output files and for diagnosing convergence of the algorithm. All other calculations were performed with S-plus.

Monitoring convergence of GS is very important step in Bayesian analysis since only the iterations after convergence are used to obtain estimates of parameter distributions (such as mean, median, standard deviation, and quantiles). For monitoring convergence, it is often recommended that several chains are generated independently for each model parameter (6). Visual examination of trace plots, autocorrelations, and Gelman-Rubin (GR) diagnostic were applied to diagnose convergence. GR uses several parallel chains with widely dispersed starting values with respect to the true posterior distribution to assess convergence. This statistic compares the variability between and within-chains by estimating a potential scale reduction factor. Approximate convergence is diagnosed when the variance between the different chains is no larger than the variance within each individual chain and their ratio is approximately equal to one (6). Based on the convergence criteria mentioned above, we generated 3 parallel runs of 10,000 iterations with dispersed starting values for each model parameter. Posterior inference was based on empirical summaries of the final 2,000 samples in each run.

As mentioned before, a Bayesian analysis results in distributions (called *posterior distributions*) for each parameter in the model. Thus, parameter estimates do not consist of just a point estimate as is the usual case, but instead consist of entire distributions that can be summarized via, for example, medians and quantiles. These quantiles can be useful for comparing distributions and for assessing changes that may occur under different modeling assumptions. Another commonly used summary is the probability that a parameter is below or above 0 which can be used instead the *p*-value used in classical statistics to determine whether a parameter is statistically significant or not.

C. Results

Convergence. For all parameters, the trace plots of the last 200 iterations for three independently generated chains demonstrated good "chain mixture" (an example is in Fig. 5.2A), an indication of convergence. Auto-correlation values across successive parameter draws ranged from 0.05 to 0.25 indicating that the realized value of a parameter in a given iteration did not depend on the sampled values in the preceding iterations (Fig. 5.2B). When the autocorrelation across sampled parameter values is low, the final sample used for estimation is closer to a random sample. GR also indicated that there was convergence of the chains (Fig. 5.2C, red line approximately equal to 1). The posterior distributions of the model parameters obtained from the sampled values reflected smooth kernel densities (Fig. 5.3A-D).

Non-informative prior distributions. Posterior point parameter estimates (posterior means) were approximately equal to the estimates generated with logistic regression analysis (12) (Table 5.1). However, elements such as the quantiles of the parameter posterior distributions (Table 5.1) and the posterior probability of positive or negative values for each input parameter (Table 5.3) are of primary interest. For example, the estimated posterior mean of the effect of the average temperature of July and August is -0.47 with a [-0.678, -0.268] 95% credible interval. Similar results would also arise from a classical statistical analysis. However, the parameter for July and August average temperature is a distribution (Fig. 5.3A) and further conclusions can be derived. For example, there is a small chance (2.5%) to have an estimate of the very low value of -0.678 or the high value of -0.268, but it is more likely that the regression coefficient is between -0.54 and -0.4 (Table 5.1). These observations lead to the conclusion that temperature in July and August is negatively associated to SSR prevalence.

Another example is the parameter of August precipitation (Fig. 5.3B). The posterior distribution and the corresponding quantiles given in Table 5.1 indicate that this parameter is most likely to be around 0 with a 2.5% probability of taking a value below -0.04 or a value above 0.058. Most of the mass of the posterior distribution (99.4%) is in the positive and only 6% is in the negative space (Table 5.3). These observations lead to the conclusion that almost certainly the effect of August precipitation on disease prevalence is positive and close to 0. This observation agrees with the results of the logistic regression analysis where we found that August precipitation was not significantly different from 0 at the 5% level of significance. Similar interpretations can be derived for all posterior parameter distributions (Tables 5.1 and 5.3).

Very informative prior distributions. Use of very informative prior distributions had an effect on the posterior distributions of the intercept, and the regression coefficients representing the effect of July and August precipitation, and state of Iowa (IA) and Ohio (OH) (Table 5.2). These parameters exhibit "sensitivity to the choice of prior" distributions suggesting that the data do not contain overwhelming information about the effect of these parameters on SSR prevalence. The most noticeable influence of the prior distributions on the parameter posterior distribution was observed on August precipitation and OH parameters.

The posterior distribution for August precipitation changed noticeably when a very informative prior distribution was used (Fig. 5.3D) in place of a non-informative prior distribution (Fig. 5.3B). With a very informative prior distribution, the posterior distribution for August precipitation was confined to the positive space (Fig. 5.3D) and the posterior mean shifted from 0.0104 (with non-informative prior distribution) to 0.1138 (with informative prior distribution).

The change in the posterior distribution of August precipitation indicates that the influence of the prior information on the form of the posterior distribution is non-negligible. Thus the choice of the prior distribution becomes important. Our assumptions for constructing the prior distribution were that the effect of precipitation during the month of August should be positive and about half of the July precipitation effect. If the sample data had been very informative for the parameter representing August precipitation then the posterior distribution should look like the one obtained using a non informative prior distribution. However, the posterior distribution changed considerably. This indicates that the analysis based on the sample data alone may not result in sufficiently accurate inference for the effect of precipitation during August on SSR prevalence.

The OH parameter has a negative posterior mean when a non-informative prior distribution is used and a positive posterior mean with a very informative prior distribution (Tables 5.1 and 5.2). Furthermore, the posterior distribution quantiles and the probability that the effect of OH is negative or positive (Table 5.3) also change significantly when different priors are used. The August precipitation and OH parameters were not selected as statistically significant ($P = 0.05$) in the logistic regression analysis, where estimation was carried out using the method of maximum likelihood that relies exclusively on sample data (12).

D. Discussion

We undertook the analysis of the SSR prevalence data from a Bayesian viewpoint to be able to derive a richer set of inferences about the effects of various factors on disease prevalence. The demonstrated sensitivity to the choice of prior distributions that was exhibited by some of the parameters in the model indicates that classical estimates, that are based solely on the sample information, should be viewed with caution. When non-informative priors were selected, the means of the posterior distributions of each of the parameters closely approximated the point estimates obtained using the method of MLE. This is to be expected, since a Bayesian analysis using non-informative priors is also based exclusively on the information that is provided by the sample data. When conducting the analysis from a Bayesian viewpoint, however, it is still possible to make a wider range of inferences about the model parameters, and more accurately assess the uncertainty associated to the point estimates.

Incorporating information about parameter values into the analysis via the choice of informative prior distributions had a noticeable effect on the estimates (both point and distributional) that were obtained for some of the model parameters. As stated earlier, this is an indication that at least with respect to some factors, the sample data offers inconclusive evidence on their effect on disease prevalence. Two factors stand out as particularly sensitive to whether extra-sample information is incorporated into the analysis or not: the effect of precipitation during the month of August, and the effect of the state of Ohio relative to the other states.

Literature on Sclerotinia stem rot indicates that precipitation around the flowering stage (9,13,15) can have a significant effect on disease occurrence. Most studies are focused on the effect of air temperature on sclerotia germination and apothecia production or SSR incidence and severity. The logistic regression analysis results (12) demonstrated that the absolute value of the average air temperature parameter was significantly higher than the value of the July total precipitation parameter (0.4 for average air temperature versus 0.029 for July precipitation), though both parameters were statistically significant ($P = 0.05$) for soybean SSR prevalence. August precipitation was not a statistically significant explanatory variable for the prevalence of SSR in the north central region of USA. In an earlier analysis (16), it was concluded that average temperature of July and August is a limiting factor for soybean SSR prevalence in the north central region of USA while July and August precipitation is not.

Results of Bayesian analysis are not in conflict with these results but rather provides more insight on the various factors on SSR prevalence. For example, our analysis suggest that the four-year data set is informative enough to give a reliable estimate of the effect of average air temperature during July and August on SSR prevalence but not for the effect of precipitation in July and August. During the four years of survey precipitation in July and August was always at least as high as the 30-year average for the north-central US. This may also explain the low parameter estimate for the effect of July and August precipitation that was obtained in logistic regression analysis (12).

Similar conclusions can be drawn for the effect of the state of OH. Only samples collected over two years from this state were available for analysis. Two years of data may not be enough to capture weather variability associated to location needed for explaining SSR prevalence in Ohio especially if we consider that only four cases of SSR were observed in this state during that period. Thus, the sample size necessary for robust estimation of the regression coefficient associated to the Ohio indicator may need to be much larger than what was used in this study. This hypothesis appears credible if we take into account the fact that prevalence of SSR was low in Ohio.

If reliable extra-sample knowledge on the effect of the explanatory variables on the biological phenomenon exists, then Bayes' theorem provides the mechanism by which that information can be incorporated into the analysis. This prior knowledge may come from experts' opinions, published experimental results or a combination of both. However, it is generally true that there is subjectivity in selecting the form of informative prior distributions, to reflect that knowledge. Notice that scientists who operated from within a classical framework also incorporated prior knowledge into experiment, but in a less formal way; in the classical paradigm, only the design of an experiment is a vehicle for inclusion of prior information.

For Sclerotinia rot, literature is available on the factors affecting SSR incidence or severity in several crops. Nevertheless, our analysis focus on SSR prevalence and use of information on SSR incidence or severity to construct prior distributions for the parameters in the SSR prevalence model may not be appropriate. Incidence, severity and prevalence are separate disease scales and magnitude of the effect of explanatory variables may differ among these disease scales. Thus, we did not feel that enough extra-sample information was available to construct very informative prior distributions for every factor in our model. Actually, the most informative prior distributions were imposed to July and August total precipitation. The choice of prior distributions for these parameters was arbitrary.

Although only a part of a more extensive analysis is presented here, several other informative prior distributions were considered, some of which did not have a clear biological interpretation but were suitable for examining the sensitivity of results to changes in the priors. It is noticeable that results were rather consistent across the wide range of prior assumptions: inferences of average air temperature of July and August were very stable, while those about the effect of July and August precipitation and Ohio state were sensitive to the choice of prior distributions. If the use of informative prior distributions improves the estimation of model parameters, then in addition it will improve predictions of SSR prevalence in the North-Central region of the United States. Prediction is a goal of modeling and is always associated to the data set used in model development. Prediction limitation due to data dependence is a topic of our current investigation and will be discussed in the future.

E. Appendix

Bayesian analysis was performed with an iterative, numerical approach called Gibbs sampler (GS). Implementation of the GS requires the specification of full conditional distributions of the parameters, i.e. the conditional distributions of each parameter given the values of all of the other parameters.

The response variable Y_i is a binary variable taking the value 1 if any of the 20 stems in field *i* was infected with Sclerotinia stem rot or 0 otherwise. Accordingly, the likelihood function has the Bernoulli form:

$Y_i \sim \text{Bernoulli}(p_i)$,

where the probability of infection p_i was modeled as a function of weather, management practices, and field location. A logit transformation was used to linearize the association of p_i and input variables.

logit (p_i) ~ a_0+a_1*[average temp]+a_2*[July prec]+a_3*[tillage]+a_4*[state]+a_5*[August prec]

a_0 ~ non-informative

a_1 ~ non-informative / informative

a_2 ~ non-informative/ very informative

a_3 ~ non-infromative/ very informative

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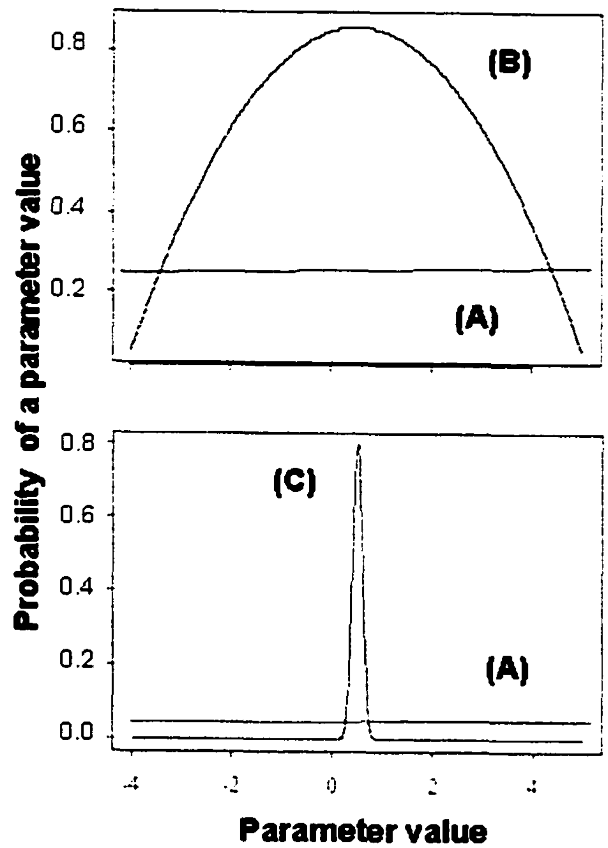

Fig. 5.1 Illustrations of non-informative (A), informative (B), and very informative (C) parameter prior distributions in Bayesian analysis.

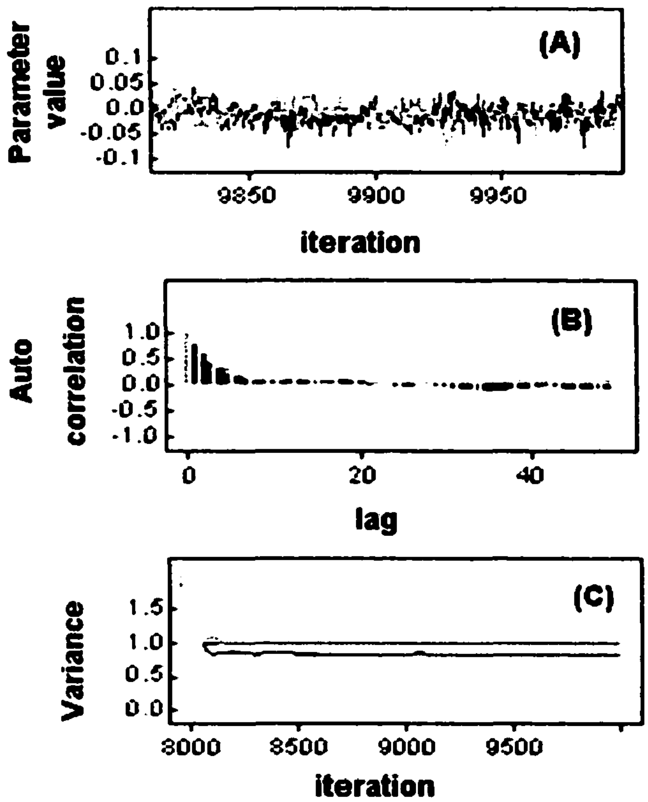

Fig. 5.2 Dynamic trace plot of no tillage indicator variable values against iteration number from three runs of the Gibbs sampler (**A**), plot of autocorrelation function of August precipitation variable out to lag 50 (**B**), and plot of the calculated Gelman-Rubin convergence statistic (**C**). Green line represents the width of the central 80% interval of the pooled runs, blue line is the width of the 80% intervals within the individual runs, and their ratio *R* (= pooled/within) is the red line. *R* is expected to be approximately one in convergence.

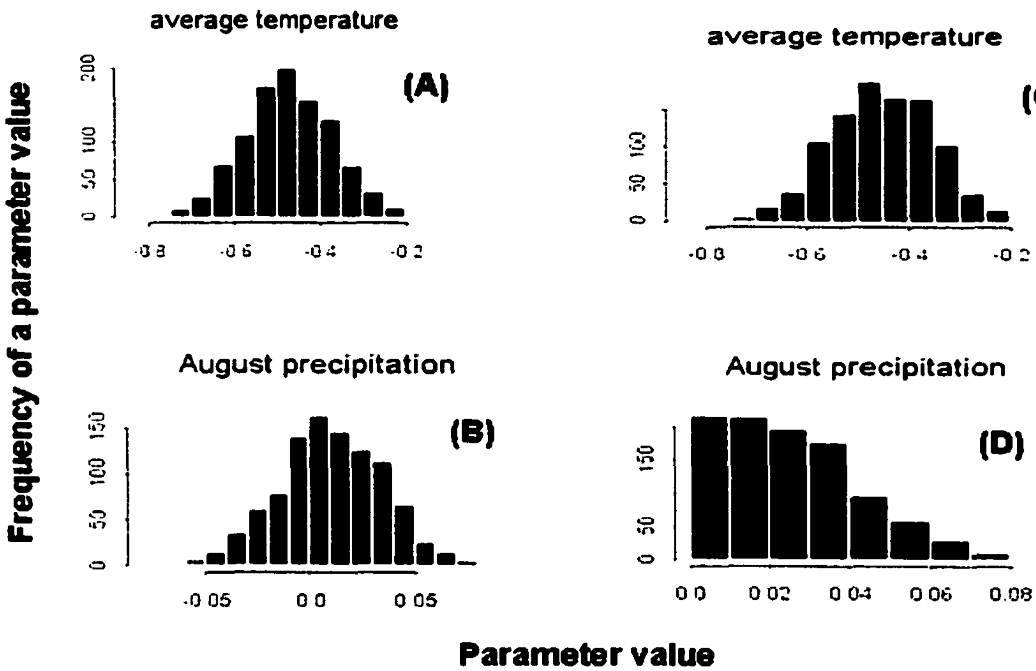

Fig. 5.3 Estimated posterior distributions of the parameter of average air temperature of July and August with non-informative (**A**) and informative (**C**) prior distributions and for the parameter of August precipitation with non-informative (**B**) and informative (**D**) prior distributions.

Table 5.1. Posterior parameter distribution summaries for Gibbs sampling using non-informative prior distributions based on iterations 8,000-10,000

Parameter	Mean	Standard deviation	Quantiles 2.5%	25%	75%	97.5%	Median
Intercept	-4.45	0.59	-5.693	-4.798	-4.059	-3.316	-4.41
Average temperature of July and August	-0.47	0.103	-0.678	-0.543	-0.404	-0.268	-0.478
July precipitation	0.039	0.021	-0.006	0.025	0.054	0.076	0.04
(No tillage)*Average temperature	-0.0104	0.022	-0.054	-0.025	0.004	0.031	-0.009
(Minimum till)*Average temperature	0.0137	0.0123	-0.009	0.005	0.021	0.039	0.014
IA	0.69	0.531	-0.239	0.334	1.028	1.837	0.652
MN	1.181	0.532	0.216	0.821	1.519	2.284	1.145
OH	-1.09	0.945	-3.178	-1.678	-0.405	0.52	-1.036
August precipitation	0.0104	0.025	-0.04	-0.006	0.029	0.058	0.01

Table 5.2. Posterior parameter distribution summaries for Gibbs sampling using informative prior distributions based on iterations 8,000-10,000

Parameter	Mean	Standard deviation	Quantiles 2.5%	25%	75%	97.5%	Median
Intercept	-7.031	0.528	-8.09	-7.353	-6.603	-5.99	-7.014
Average temperature of July and August	-0.432	0.111	-0.669	-0.524	-0.372	-0.241	-0.43
July precipitation	0.1685	0.0173	0.133	0.157	0.18	0.203	0.168
(No tillage)*Average temperature	-0.0134	0.0187	-0.05	-0.026	-0.001	0.024	-0.013
(Minimum till)*Average temperature	0.0052	0.0136	-0.019	-0.003	0.015	0.035	0.0047
IA	0.901	0.289	0.267	0.525	1.092	1.366	0.883
MN	1.353	0.43	0.431	0.796	1.638	2.042	1.33
OH	0.044	0.0357	-0.024	0.016	0.066	0.107	0.0437
August precipitation	0.1138	0.021	0.073	0.096	0.133	0.156	0.113

Table 5.3. Probabilities of parameters to be negative or positive using non-informative, and very informative prior distributions

	Non-informative prior distributions		Very informative prior distributions	
Parameter	*Pr(< 0)*	*Pr(> 0)*	*Pr(< 0)*	*Pr(> 0)*
Intercept	1.0	0.0	1.0	0.0
Average temperature of July and August	1.0	0.0	1.0	0.0
July precipitation	0.036	0.957	0.0	1.0
(No tillage)*Average temperature	0.672	0.306	0.762	0.216
(Minimum till)*Average temperature	0.129	0.855	0.311	0.667
IA	0.320	0.662	0.0	1.0
MN	0.887	0.113	0.0	1.0
OH	0.088	0.912	0.127	0.867
August precipitation	0.006	0.994	0.0	1.0

CHAPTER VI. IMPACT OF MANAGEMENT PRACTICES ON REGIONAL PREVALENCE OF SOYBEAN SCLEROTINIA STEM ROT IN THE NORTH-CENTRAL REGION OF THE UNITED STATES AND ON FARMERS' DECISIONS UNDER UNCERTAINTY

A paper accepted with revision by Plant Disease

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A. Abstract

Regional prevalence of soybean Sclerotinia stem rot (SSR), caused by *Sclerotinia sclerotiorum* (Lib.) de Bary, was modeled using management practices (tillage, herbicide, manure and fertilizer application, and seed treatment with fungicide) and summer weather variables (mean monthly air temperature and precipitation for the months of June, July, August, and September) as inputs. Logistic regression analysis was used to estimate the probability of stem rot prevalence with disease data from four states of the North-Central Region of U.S. (Illinois, Iowa, Minnesota, and Ohio). Goodness of fit criteria indicated that the resulting model explained well the observed frequency of occurrence. The relationship of management practices and weather variables with soybean yield was examined using multiple linear regression ($R^2 = 0.27$). Variables significant to SSR prevalence, such as temperature of July and August, precipitation of July, tillage, seed treatment, liquid manure, fertilizer, and herbicide applications were also associated with high attainable yield. The results suggest that SSR occurrence in the North-Central Region of the United States is associated with environments of high potential yield. Farmers' decisions about SSR management, when the effect of management practices on disease prevalence and expected attainable yield is taken into account, are examined. Bayesian decision procedures were used to combine information from our model (prediction) with farmers' subjective estimation of

SSR incidence (personal estimate, based on farmers' previous experience on SSR incidence). *MAXIMIN* and *MAXIMAX* criteria were used to incorporate farmers' site-specific past experience with SSR incidence, and optimum actions derived using the criterion of profit maximization. Our results suggest that management practices would be applied to increase attainable yield despite their association with high disease risk.

B. Introduction

In recent years, Sclerotinia stem rot of soybean (SSR), caused by *Sclerotinia sclerotiorum* (Lib.) de Bary, has emerged as a leading cause of soybean yield losses in the North-Central soybean production region of the United States. Before 1990's, the disease was known to cause only localized epidemics in Michigan, Minnesota, and Wisconsin (20) where soybeans of maturity groups 0 to I are grown. In the same region, SSR was ranked 12th as a cause of yield losses in 1990 (11) and second in 1994 (55). In the 1996 growing season, the disease was in epidemic proportions in Iowa (53), much more severe than in 1992 and 1994 (57).

Management practices that are intended to increase soybean yield (such as narrow row spacing, increased plant populations, early planting dates and high soil fertility), reduced tillage, or rotation with susceptible crops are factors that increase the soil inoculum density and may also contribute to the increased SSR occurrence in the North-Central Region of the U.S. (12,19,27,33,42). Finally, above-normal precipitation and low temperatures occurring during the critical infection period of flowering also favor SSR development (53).

Deep plowing has been recommended for control of white mold (51). In a study (42) on dry edible beans it was shown that a 3-year rotation did not reduce sclerotium populations significantly, suggesting that attempts to manipulate sclerotial populations may have limited effect on either soilborne or airborne inoculum density. No-tillage significantly reduces SSR occurrence (27,53). Fewer apothecia have been found in no-tillage (16,27). Fertilizers have been found having contradictory effects on *S. sclerotiorum*: in one study they did not affect sclerotium germination (42), while in another (12) fertilizer increased significantly the number of produced apothecia. Manure applications were positively correlated to SSR incidence (36) and increased carpogenic germination has been observed in soils with high organic matter content (13).

Planting in wide rather than in narrow row widths (45) or at low plant populations (21) has been suggested as a successful management practice of SSR control since it reduces canopy density. Dense canopy creates favorable humidity and temperature conditions for SSR development within the canopy (5,6). Treatments of sunflower seeds with benomyl, improdione, or vinclozolin eliminated seed-borne *Sclerotinia sclerotiorum* and increased the number of surviving seedlings (22). The fungus uses the seed as a nutrient base to produce sclerotia in soil, and thus establishes itself in new fields (1,49). For the reason, seeds infected with mycelia may serve as means of *S. sclerotiorum* dissemination over long distances (1).

Increase in Sclerotinia stem rot incidence in the North-Central Region of the United States is associated with yield losses and it is of concern for two reasons: the scarcity of resistant cultivars in the maturity groups appropriate for the region and the cost of fungicides for control of Sclerotinia stem rot (18,27,34). Sclerotinia stem rot can be controlled successfully by fungicides in susceptible crops such as dry bean and canola (46) but chemical control of SSR in soybean has not proven economically feasible (17,27). Complete resistance to SSR has not been reported (17,19,27) and only recently have cultivars with some resistance to SSR been planted in the region. As a result, current strategies for controlling SSR in soybean emphasize the use of management practices that reduce canopy density (46).

Farmers are interested in profit or net return from farming (48). Most farmers do not try to maximize yield while ignoring costs, but expenses involved in crop production and disease management are also included so that the net return from crop production can be calculated (48). If net return is the sole criterion used by a producer to choose between two or more alternatives, then the one with the highest expected monetary value (EMV) would be the one chosen (2,48). Farmers, however, may have different preferences for certain practices, given individuals' current situation, the EMV for a given practice, and the perceived probabilities for a situation (e.g. SSR incidence level in a field). Economists use the concept of utility to encapsulate such information and if individuals' utilities are known, one can attempt to optimize the expected utility instead of EMV (2,48).

Several studies on the risk and profitability of different soil-conserving tillage practices have been conducted but the results are rather inconsistent. Some studies suggest that conservation tillage is the most profitable system although the most uncertain about expected yield (28), while others show that average net returns per acre are higher for conventional tillage systems compared with other tillage systems (14). Oplinger and Philbrook (37) suggested that soybean seeding rates in no or reduced tillage should be 15 to 32% higher than these used in conventional tillage so that equivalent yields are to be expected. Narrow row spacing is mainly for weed control but it is also associated with high plant populations and high potential yields (25,38). Seed treatments with Benlate, Captan, Vitavax, and Topsin have been reported to contribute to higher germination and yield than non treated seeds (43). In another study seed treatments were associated with reduced cultivar susceptibility to seedling diseases due to protection against soilborne pathogens and improved seed quality (31).

Studies by Savary *et al.* have quantified the effects of management practices on yield and disease incidence in large geographic scale (40,41). These studies developed protocols for characterizing patterns of rice cropping practices and showed the potential for developing pest management strategies that can be adapted throughout tropical and subtropical Asia, rather than being site-specific. Sclerotinia stem rot in the U.S. north-central production system provides a model system for this type of study because of the voluminous data collected on the disease in this region (52-54).

Objectives of our study were: a) to quantify the effect of input production variables on SSR prevalence; b) to investigate the relationship between yield and production variables that affect SSR occurrence; and c) to examine the effect of soybean farmers' production decisions on SSR incidence using decision theory under uncertainty.

C. Materials and methods

Data collection. In 1995 and 1996, soil and soybean stem samples were collected sometime between the last week of September and the first week of November from 1,155 fields in Illinois, Iowa, Minnesota, and Ohio by the National Agricultural Statistical Service (NASS). In total, 352 fields from Illinois, 398 from Iowa, 220 from Minnesota, and 185 from Ohio were collected. The soybean fields sampled in 1996 were different from those sampled in 1995 because of the corn-soybean rotation schemes in the Corn Belt. Details on the method used for field selection have been reported elsewhere (54). We describe data collected as below: a) *SSR incidence data*. 20 stems were collected from each sampled field in a zigzag pattern. Stems were externally observed for presence or absence of lesions. The stems were also longitudinally split and checked for presence or absence of sclerotia in the pith. For stems with signs and symptoms that could not be readily identified, isolation was made on acidified potato dextrose agar for further verification. SSR was found in 58 of the 1,155 sampled fields (2 in Illinois, 2 in Ohio, 35 in Iowa, and the rest in Minnesota). The disease incidence was lower than 16% in 84.5% of the cases where SSR was found; only in one case was the disease incidence very high (80%). In this study the term prevalence is used to describe the percentage of fields in which the disease was found, and the term incidence is used to describe the percentage of infected soybean plants sampled in a field. b) *Soybean yield data.* In each field, NASS had established and maintained two yield-assessment plots. Yield data used in this study are the average yield of the two assessment plots. c) *Soil texture data.* Soil was collected from each field, and soil texture was determined with methods previously described (56). d) *Management practices data.* NASS enumerators interviewed farmers about management practices (irrigation, tillage, herbicide, manure and fertilizer application, and seed treatment with fungicide) that had been used in the sampled fields. e) *Weather data.* Mean monthly air temperature and precipitation for the months of June, July, August, and September were obtained from the National Oceanic and Atmospheric Administration (NOAA) through the National Climatic Data Center (NCDC) in Asheville, NC. For each sampled field, mean monthly temperature and precipitation were obtained from the nearest weather station. In 82% of the cases the sampled fields were located less than 20 km from the nearest weather station. Only in 18 cases in Illinois, 8 in Iowa, 7 in Minnesota and 11 in Ohio was the distance between field and weather station greater than 20 km. SSR was not found in any of the fields located in distance greater than 20 km from the nearest weather station.

Correlation between variables. *a) Continuous variables* were tested for possible correlation (using the CORR procedure in SAS; Statistical Analysis Systems: SAS Institute, Inc., Cary, NC). *b)* Two-way contingency tables were used to examine any possible associations between the *categorical variables* used in the models. The null hypothesis of independence between any two categorical variables was tested using a chi-square test. *c)* To check the possibility of association between *continuous* and *categorical variables* the null hypothesis of no difference between the means of the continuous variable within each category of categorical variables was tested using a *t*-test at 95% level of significance. In this case, the values of each categorical variable (two for all categorical variables, except the case of manure application where the values are three) are equivalent to different treatments used in an experimental design. Collinearity among predictors is important and should be investigated before any quantification takes place, since it may affect the efficiency of estimated parameters.

SSR prevalence model. Logistic regression was used to identify factors significantly associated with the regional prevalence of SSR. If the dependent variable Yi is the absence ($Yi = 0$) or presence ($Yi = 1$) of the disease in the ith field, the probability of disease presence is:

$$P(Yi = 1) = \exp\{a + \Sigma b_j X_j\} / (1 + \exp\{a + \Sigma b_j X_j\}) . \qquad (1)$$

where $i = 1, \ldots, n$ and $j = 1, \ldots p$.

Here, a and $b_i \ldots$, b_j are parameters to be estimated, and the X_j's are the covariates or predictors. They are similar to the intercept and regression coefficients in an ordinary multiple regression model. However, their interpretations are somewhat different from these in logistic regression (23). LOGISTIC procedure in SAS was used to estimate parameters in model (1). The numbers of concordant and disconcordant pairs and correlation indices such as Somers' D, Gamma, and c (equivalent to R^2 for linear regression analysis) were used to select the best fitted model. A pair is called *concordant* when the observation with the larger ordered value of the response has a lower predicted event probability than the observation with the smaller ordered value of the response. A pair is called *discordant* when the observation with the larger ordered value of the response has a higher predicted event probability than the observation with the smaller ordered value of the response. A model fit well the data when the proportion of concordant pairs is high and the proportion of disconcordant pairs is low. The correlation indices are computed from the numbers of concordant and discordant pairs of observations. A model with higher values for these indices has better fitness than a model with lower values for these indices.

Soybean yield quantification. Linear multiple regression analysis was used to quantify the effects of weather variables and management practices on yield. First, separate stepwise procedures were used for assessing the relationships between the independent and dependent variables. Then a multiple regression model was selected using backward elimination procedure. Starting from a saturated (or full) model, we sequentially dropped covariates or predictors not significantly associated with the response variable. Criteria used to evaluate the model were the graphical appraisal of randomness and normality of the residuals, coefficient of determination (R^2), and F-value (35). In this study, regression analysis was used to describe the relationship between the variables that contribute to SSR prevalence and yield rather than to develop a model for yield prediction.

Input variables. Variables are listed in Table 1. *Disease model.* Average air temperature of July and August, precipitation from June to August, no-tillage, minimum tillage, and conventional tillage, percentage of soil sand, clay, and silt, seed treatment, herbicide, manure, and fertilizer application were used as input variables for the disease model. *Yield model.* Air temperature of July, August, and September, precipitation of June, July, August, and September, tillage practices, percentage of sand, clay and silt in the soil, seed treatment, herbicide, manure, and fertilizer application were used as input variables for the description of yield variation.

Irrigation was used in 8 fields (3 in Illinois and 5 in Iowa) in 1995 and in 3 fields (1 in Illinois, 1 in Iowa and 1 in Minnesota) in 1996. SSR was not found in any of the irrigated fields. Because of the very small number of irrigated fields (13 out of the 1,155 sampled fields) that generated technical difficulties in the analysis, irrigation was not included in the final analysis. Tillage systems were classified into three categories according to the amount of surface residue (3). Conservation tillage systems maintain greater than 30% surface residue after planting. Tillage practices that maintain 15 to 30% surface residues are categorized as minimum-till, whereas those that maintain less than 15% surface residue are classified as conventional till. There were 331 fields in no-tillage, 409 in minimum till, and 415 in conventional tillage in 1995 and 1996. We considered two types of manure application: dry broadcast with or without incorporation and liquid broadcast with or without incorporation. Source of manure were beef, hogs, pigs, and dairy. Fertilization included K_2O application (100% of cases where fertilizer was applied), P_2O_5 and N application (72% and 12%, respectively, of the cases where fertilizer was applied). Fungicides used in seed treatments were not available and could have been applied by seed companies or interviewed farmers.

There were two differences between the prevalence and the yield models concerning the input variables. a) The yield model included September air temperature and precipitation, since previous studies showed the importance of these variables to yield (11, 20), while the SSR prevalence model excluded September air temperature and precipitation, since in fall the disease had already occurred. b) In the yield model, tillage practices were used as one categorical variable with conventional tillage as the reference group. In the SSR prevalence model, no-tillage, minimum tillage, and conventional tillage were considered as separate categorical variables. No-tillage was used as implementation or not, minimum tillage as even or odd number of passes, and conventional tillage as number of passes (one, two, three, or more than three). The reason for this differentiation in the use of tillage in yield and SSR prevalence models stems from studies on adoption of no-till technology that have shown that there is delay in no-till adoption primarily because of the high cost of replacing the existing conventional planter and of the cost of learning how to obtain high crop yield with no-till technology (26). Thus, even high risk of SSR might not provide enough motivation to farmers for changing tillage to reduce SSR risk. However, farmers may still be willing to modify the chosen tillage (e.g. change the number of passes in minimum tillage) to differentiate the expected disease risk.

Decision theory under uncertainty. Two *decision criteria* are usually used when a farmer's behavior under uncertainty is examined: a) profit maximization, which is the most common assumption made in the production economics literature and government stabilization policies, and b) utility maximization where it is hypothesized that farmers maximize their expected "utilities" (satisfaction) (2,29,44). Utility maximization does not exclude profit maximization but rather includes it as a special case. Profit maximization was used in the present study.

The decision theory involves enumerating all possible payoffs and selecting the action that provides the best expected payoff. This procedure can be described as:

$$E(U(\alpha, \theta)) = \max [\, \Sigma\, U(\alpha, \theta)*P(\theta)\,] \qquad (2)$$

where α denotes the management option, θ is SSR incidence in a field, $E(U)$ stands for expected utility (the expected profit given a chosen management practice and a level of SSR incidence). If no information about SSR incidence is available, a farmer will choose the management practice that maximize his utility $U(\alpha, \theta)$ given the probability $P(\theta)$ of SSR incidence. Since no information for θ is available, $P(\theta)$ is the decision-maker's subjective estimation of SSR incidence deriving from personal experience (8).

The farmer receives further information about the expected SSR prevalence in his area through a forecaster z (SSR prediction model). The farmer updates his estimation θ combining his past experience with the information of the forecaster via Bayes' theorem:

$$\underset{\text{updated estimation}}{P(\theta \mid z)} \;\propto\; \underset{\text{past experience}}{P(\theta)} \;*\; \underset{\text{forecast}}{P(z \mid \theta)}\ . \qquad (3)$$

Then, substituting the updated estimation of equation (3) in equation (2) we have:

$$E(U \mid \theta) = \max [\, \Sigma\, U(\alpha, \theta)*P(\theta \mid z)\,]\ . \qquad (4)$$

Empirical data about how farmers interpret regional disease risk into within field incidence are not available. It is suggested (58) that farmers weight their past experiences giving more weight to recent observations and less weight to those that occurred in some time in the past. In the present case, past experiences are incorporated using the *MAXIMIN* or the *MAXIMAX* criterion (39). The *MAXIMIN* criterion corresponds to farmers with prior experience indicating that their fields should be the ones under high SSR risk (e.g. farmers' that experienced severe SSR infection the proceeding year). The *MAXIMAX* criterion corresponds to farmers with prior experience indicating that their fields should be the ones under low SSR risk (e.g. farmers with no past experience with SSR) (39). *Payoff tables* are used to represent $E(U)$, net profit, under different management practices -SSR scenarios (30,39). In pay-off tables rows depict management options, and columns show expected net profits under different SSR incidence levels.

In our study we used four management practices (tillage, manure application, seed treatment, and combination of manure and seed treatment). For each management practice, we considered two options: apply the management practice or not, and three SSR incidence levels (0, 10, and 60%). Iowa is used as an example but a similar approach can be derived for all states. Expected SSR prevalence and yield with or without the management practices were estimated using the parameter estimates of Table 4. The cost of manure application was obtained from the Department of Agricultural and Biosystems Engineering, ISU, the cost of seed treatment from Gustafson, TX, and the soybean price as a 10-year average.

D. Results

Correlation between variables. Three types of correlations were used in our analysis: a) *Correlation between continuous variables.* There were significant negative correlations between percentage of sand and percentage of silt, and between percentage of sand and percentage of clay, positive correlation between air temperature of July and August, and negative correlation between temperature and precipitation of September. Due to these correlations, only the percentage of clay, September precipitation, and the average air temperature of July and August were selected as inputs in the models with management practices. After these modifications, remaining correlations between the continuous variables were not higher than 0.14. b) *Correlation between categorical variables.* There were significant positive linkages between the categorical variables (management practices) in most cases (Table 2). These linkages show a pattern in soybean production whereby farmers apply management practices in specific combinations such as herbicides, seed treatment and manure were applied mostly in minimum and no tilled fields, in Iowa and Ohio farmers that used treated seed, they applied manure and fertilizer in their fields too. c) *Correlation between continuous and categorical variables.* Most of the categorical variables (management practices) were significantly (95% level of significance) correlated with the average air temperature of July and August, and August and September precipitation (Table 3). These correlations stem from the fact that there are differences among geographical regions with regard to applied management practices (e.g., most no-tilled fields are located in Illinois and most minimum-tilled fields are located in Minnesota). Correlations between categorical, and continuous and categorical variables were not used as a criterion to exclude variables from the model. These associations were used to interpret the results of the linear regression analysis.

SSR prevalence model. Seed treatment, application of liquid manure, and no-tillage or even versus odd number of passes in minimum tillage were significant for all states. Fertilizer application in Illinois and Minnesota and weed cultivation in Minnesota were significant. The intercept was not significantly different from zero ($P = 0.7$). SSR prevalence models for each state are in Tables 4-7, where only significant input variables ($P < 0.1$) are presented. Proportions of concordant and discordant pairs of fitted model were 88.7% for concordant and 11.0% for discordant pairs. The indices Somers' D, Gamma, and c were 0.78, 0.78, and 0.89, respectively.

Soybean yield model. For all states, summer weather variables (average air temperature of July and August, and precipitation of June, July, August and September), management practices, and interactions between management practices and weather accounted for 27% of yield variation. Low temperatures in July an August and high precipitation from June to September favored increased attainable yield. Application of herbicides and liquid manure increased yield. No or minimum tillage reduced attainable yield comparing to conventional tillage. Disease incidence (percentage of soybean plants infected in individual fields) was negatively associated with yield depending on the average air temperature of July and August. Fields notified as "Highly Erodible (HEL)" were less yielded than the rest. Linear regression parameter estimates for significant input variables ($P < 0.1$) are presented in Tables 4-7. Plots of residuals against values of input variables or predicted values showed scattered patterns.

Simulation of SSR prevalence and yield quantification. Examples of predicted disease prevalence and yield quantification generated with the parameter estimates (Tables 4-7) are in Figs. 1-3. SSR prevalence was higher in minimum-till than in no-till fields when July precipitation was less than 7.5 cm, although with higher precipitation no-till fields could have lower or higher disease risk than minimum-tilled fields depending on the number of tillage passes (Fig. 1A). Manure application shifted up all the lines of SSR incidence (not shown), implying increased risk under any tillage regime after manure application. There was a difference in the expected yield between minimum and no-tilled fields, with minimum-till superior to no-till depending on June precipitation (Fig. 1B). This difference becomes even larger if manure is applied (about 268 Kg/ha). Figure 2 shows the effects of herbicide (A) or no herbicide application (B) under no-till on SSR prevalence and yield (C) in Minnesota. No-tilled fields with applied herbicides had higher probability of SSR than fields without herbicide application in low July and August temperatures. In contrast, the SSR prevalence was expected to be lower in fields with applied herbicides than fields without, especially if July and August temperature and July precipitation was high. Attainable yield (even when SSR incidence was high, 60%) was higher with herbicides than without when average July and August temperature is 22 to 24 $^{\circ}$C and July precipitation 9 to11 cm. On the other hand, the herbicide effect was reversed in hot summers. However, in this case the probability of SSR prevalence was low.

In sandy soils the SSR prevalence was slightly higher for non-treated seed (Fig. 2B) than for treated seed (Fig. 3A) when July precipitation was high. However, there was no significant difference on the expected prevalence when July precipitation was low. Similarly, in clay soils there was no significant difference in the expected prevalence between treated or non treated seed for any precipitation range. In sandy and clay soils, however, expected yield was higher with treated seed than with no-treated (Fig. 3B).

Decision under uncertainty. Farmers who frequently or recently have observed SSR in their fields would likely decide under the *MAXIMIN* criterion considering that the worst scenario will occur: their fields will be of the highest SSR risk, even if predicted SSR prevalence in their area is low. For example, expected prevalence when manure and treated seed are used is high (0.8), but much lower when these practices are not applied (0.3) (Table 8). Farmers of the *MAXIMIN* group consider their fields to be among the 8 out of 10 fields with high SSR incidence if manure and treated seed are used or among the 3 out of 10 fields with high SSR incidence if manure and treated seed are not used. For these farmers profit is maximized when management practices are applied (Table 8, choose row yes for management practices and column 10 or 60% for incidence).

Farmers with no or rare previous SSR records in their fields will make the most optimistic choice: no SSR will be found in their fields even if predicted SSR prevalence in their area is high. These farmers consider themselves belonging to the low risk group (3 out of 10 fields with SSR if manure and treated seed are not used or 8 out of 10 fields with SSR if manure and treated seed are used) and will choose management practices to maximize their profit (Table 8, choose row no for management practices and column 0% for incidence).

E. Discussion

Our results suggest that SSR is a disease associated with management practices and weather conditions that are favorable to high attainable yield. Management practices such as manure, fertilizer, and herbicide application are associated to high attainable yield and SSR prevalence (Table 9). Frequent SSR occurrence in fields with high yield potential has been suggested in other studies as well (19,27). However, this association has been attributed mainly to factors such as narrow planting rows and high plant populations that promote dense soybean canopy (25). Dense canopy creates high moisture and low temperature within the canopy, conditions favorable for SSR development (5,6).

Low temperature of July and August, and high July precipitation are associated with increased soybean yield. This finding agrees with previous economic and agronomic studies. A study using historical yield data for the period 1891 to 1973 for the states of Iowa, Illinois, Indiana, and Missouri found that the expected yield during a drought period was less than that for a wet period (4). Soybean yield models developed by the Climatic and Environmental Assessment Services (CEAS) for Illinois, Indiana, and Iowa predict that high temperatures and low soil moisture during summer would decrease yield (15, 29). This temperature-moisture combination has been reported to be favorable for SSR development in soybean (6,19,32).

SSR prevalence is higher in minimum tillage comparing to conventional tillage (32), although the risk varies with the number of tillage passes (Fig. 1A), and is lower in no-tillage comparing to conventional tillage. Kurle *et al.* (27) observed fewer apothecia in no-tillage than in plowed fields but he concluded that differences in SSR incidence among tillage systems is mainly attributable to differences in observed plant stand and canopy density. Similarly, Gracia-Garza et al. (16) suggested that environmental and microclimatic conditions in reduced tillage, such as high soil moisture, low soil temperature and high nutrients availability are inductive to SSR development.

Lower attainable yield is found in reduced or no-tilled fields than conventional fields, although the overall effect depends on amount of June precipitation. It is positive when June precipitation is low and negative when June precipitation is high. These results agree with previous studies (24,26). Higher yield was found with no-till than conventional till in dry years probably because of conservation of more soil moisture in no-till fields than in conventional fields (24). It has been reported (27) that canopy development in no-tillage is poorer than in conventional tillage which should be a contributing factor to the yield differences observed among different tillage systems.

Application of liquid manure increases SSR risk and soybean attainable yield. Farmers in Michigan have observed higher disease incidence in areas where liquid manure was applied than the rest of the state (36). Field experiments in Iowa showed that the number of apothecia was higher in plots where liquid manure had been applied than in non treated plots (36). Also, Ferraz et al. (12) observed abundant apothecia production in high organic matter soils. It is plausible that the effect of manure is indirect since manure promotes vigorous plant growth that created a favorable for infection environment under the canopy. We draw the attention that the results concern liquid manure application. Similar relationship was not found for dry manure application.

Seed treatment is negatively associated with disease prevalence, but its effect depends on the percentage of clay in the field. Fungicidal seed treatments may prevent the dissemination of *S. sclerotiorum* in new fields (22,56). Seed treatment results in better stand establishment by preventing seedling disease problems, especially in wet conditions, than non treated seeds (13,31), consequently resulting in dense canopy favorable to SSR development (5,42,45).

In Illinois, fertilizer and its interaction with August precipitation are significant to SSR prevalence. Based on the 30-year average (1961-1991) for August precipitation in Illinois, the overall fertilizer effect on SSR prevalence is positive and negative on attainable yield. In Minnesota, based on the 30-year average temperature of July and August the effect of fertilizer on SSR prevalence and on attainable yield was negative, while the herbicide application effect was positive on SSR prevalence and on the attainable yield.

Fertilizer applications were found to have no effect or increased slightly sclerotia germination (51), while in another study (12) fertilizer increased significantly the number of produced apothecia. The potential effect of fertilizer applications on SSR development is attributed to the vigorous plant growth and dense canopy (12,51) Our results indicate that fertilizer effect on SSR occurrence is inconsistent among states. Herbicide applications (e.g. Lactofen) have been reported to reduce SSR incidence (7,10). Less closure in canopy and hypersensitive response-type mechanisms have been proposed as explanations for the herbicide application effect (7,10). However, in our study herbicides were shown to increase SSR risk in Minnesota. Since the exact applied herbicides are not known we cannot conclude whether this result is an artifact of the data set or not. Fertilizer and herbicide applications are generally associated to high potential yield. Thus, the negative relationship found between fertilizer application and yield in Illinois and Minnesota cannot be justified, but for the case that fertilizers were applied only in low potential yield fields in these two states.

There are two points to be addressed in the present study. First, weather data were collected from weather stations located in considerable distance for some fields and thus these weather data do not describe the precise environmental conditions experienced by the soybean plants. This might be one of the reasons of the low R^2 in the yield quantification. However, such macro-scale weather data have been used in studies to quantify yield (15,20,29) or to describe regional patterns (9,47,50) with good results. Our objective was only to describe the regional SSR pattern and examine if there is truly association between frequency of SSR occurrence and high yield. Second, information about presence or absence of *S. sclerotiorum* in individual sampled fields was not available. Thus, the analysis was based on an assumption that *S. sclerotiorum* was present in each of the sampled fields with equal probability.

We concluded that if decision is based on profit maximization and farmers weight highly their past experience with the disease, they will apply the management practices that maximize their profit, and thus increase the risk for SSR occurrence, since high-yielding management practices are also inductive to SSR development. Nevertheless, these conclusions are drawn neglecting the effect that these management practices may have on SSR incidence. Profit is much lower when SSR incidence in a field is 60% than 10% (Table 8). Under this perspective, farmers may follow a different decision pathway. In economic terms this means that farmers decide using the mean-variance criterion: they are willing to sacrifice part of their expected income to reduce the variance in income generated by different SSR incidence levels (8). Then utility rather than profit maximization is an accurate predictor of a farmer's decision (30). Future investigation is needed on the usefulness, if any, of macro-scale measures such as disease prevalence in site-specific decisions.

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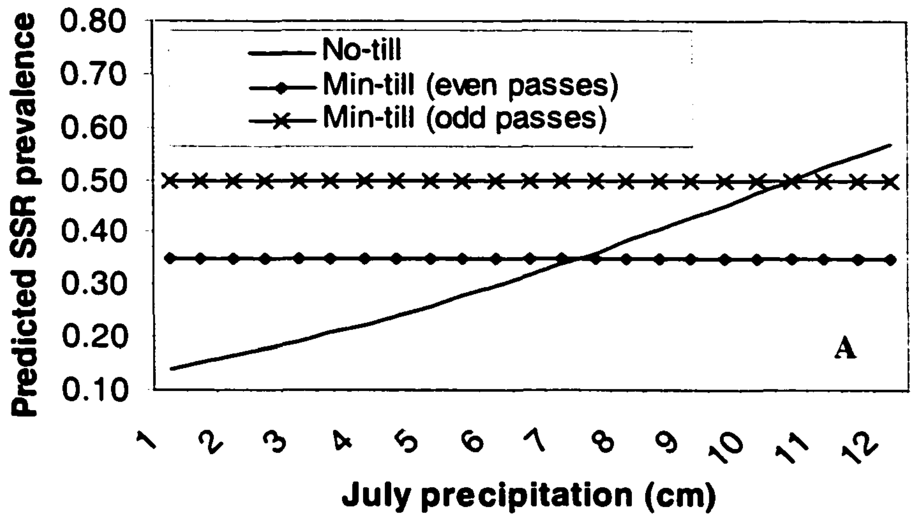
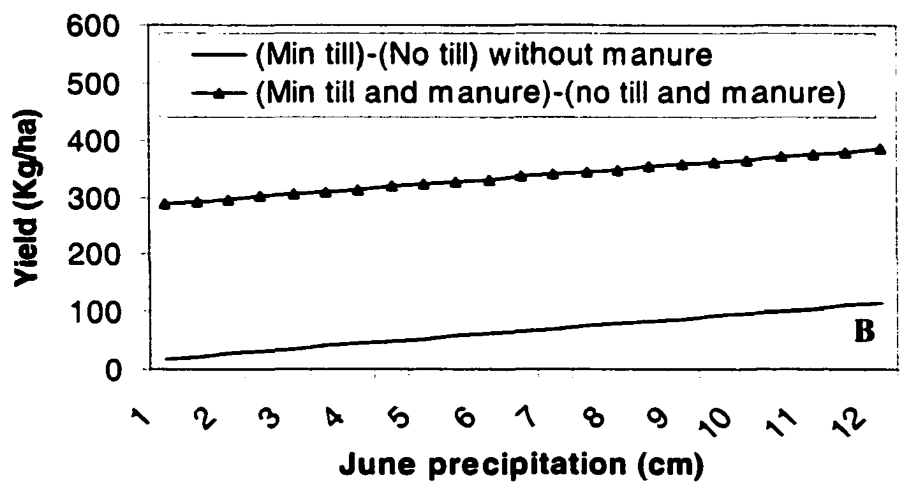
Fig. 6.1. (A) Estimated Sclerotinia stem rot (SSR) prevalence caused by *Sclerotinia sclerotiorum* in no-tilled or minimum-tilled (even and odd passes) soybean fields using the parameter estimates of Tables 3-6. **(B)** Difference in expected yield between no-tilled and minimum-tilled fields with or without manure application calculated using parameter estimates for Iowa yield quantification (Table 3). For the calculations summer months' precipitation was considered equal to 6 cm, average temperature of July and August equal to 20 ^{o}C, and clay 30%. Other management practices than manure were not considered.

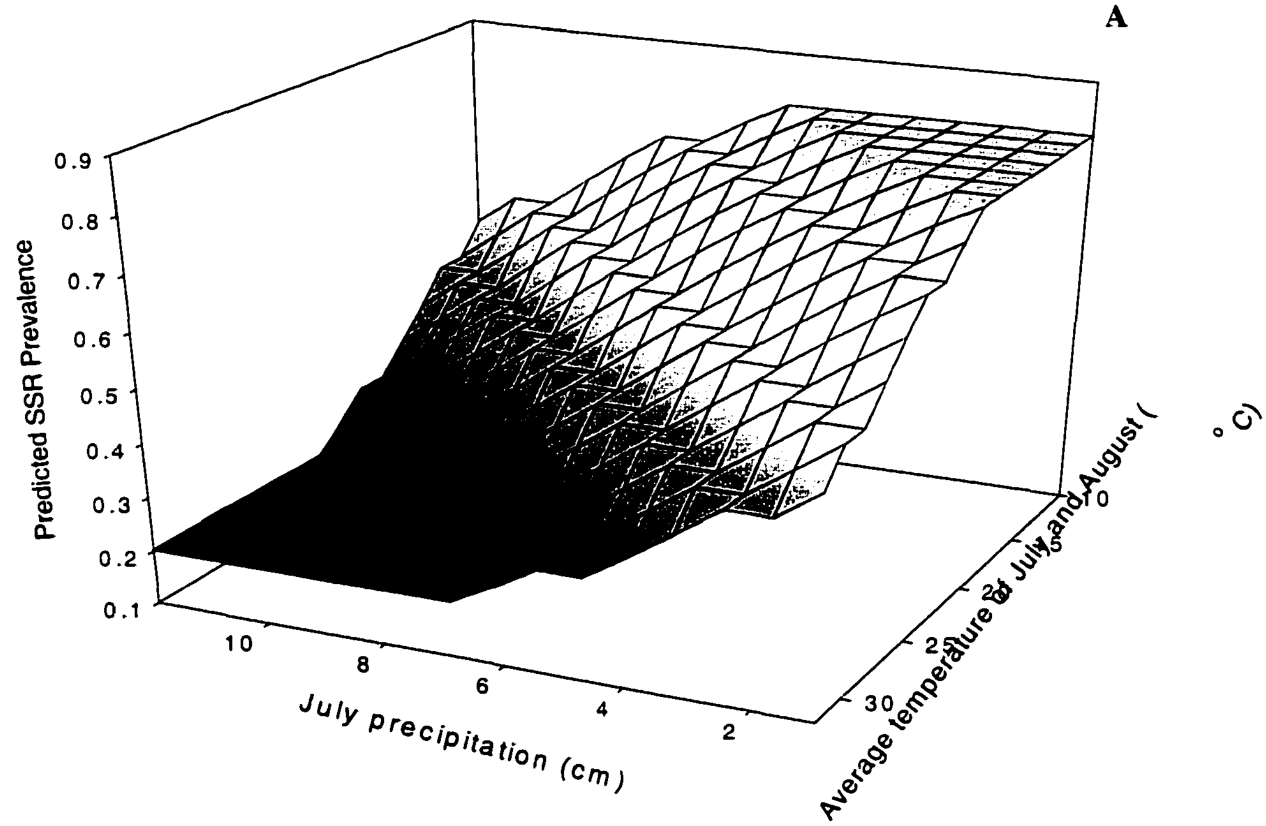


0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
Predicted SSR prevalence
No-till
Min-till (even passes)
Min-till (odd passes)
A
1
2
3
4
5
6
7
8
9
10
11
12
July precipitation (cm)

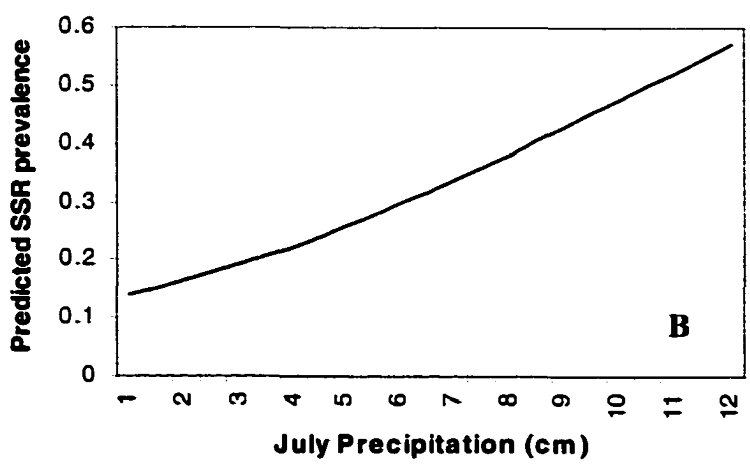


600
500
400
300
200
100
0
Yield (Kg/ha)
(Min till)-(No till) without manure
(Min till and manure)-(no till and manure)
B
1
2
3
4
5
6
7
8
9
10
11
12
June precipitation (cm)

Fig. 6.2. Estimated Sclerotinia stem rot (SSR) prevalence caused by *Sclerotinia sclerotiorum* in no-tilled soybean fields in Minnesota with **(A)** and **(B)** without herbicide application using the parameter estimates of Table 6. **(C)** Yield calculated using parameter estimates for Minnesota yield quantification (Table 6) in no-tilled fields with **(C1)** and without **(C2)** herbicide application and 60% SSR incidence. For the calculations summer months' precipitation was considered equal to 12 cm, and clay 30%. Other management practices than weed cultivation were not considered.

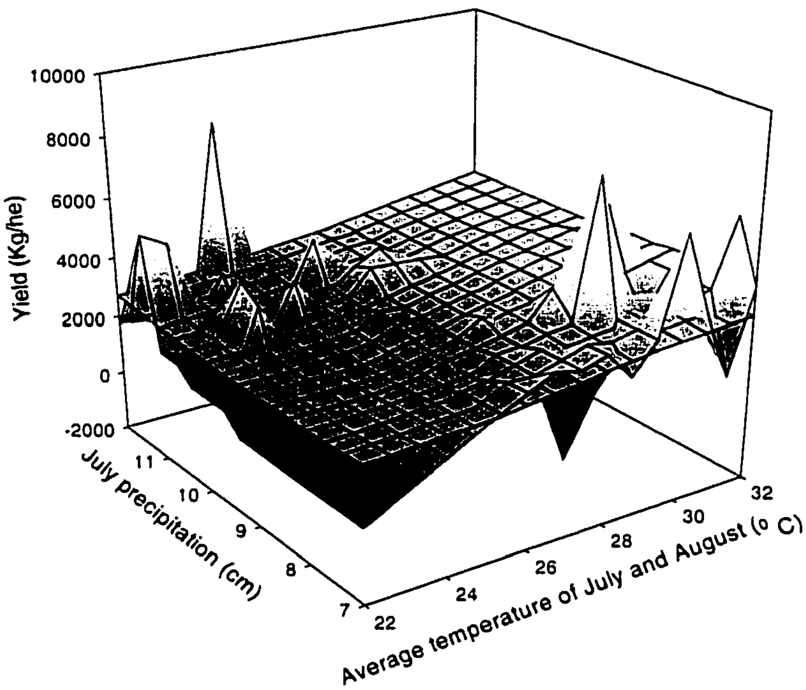

Predicted SSR Prevalence
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
July precipitation (cm)
10
8
6
4
2
Average temperature of July and August (°C)
30
0

A


Predicted SSR prevalence
0
0.1
0.2
0.3
0.4
0.5
0.6
July Precipitation (cm)
1
2
3
4
5
6
7
8
9
10
11
12

B

(C1)


10000
8000
6000
4000
2000
0
-2000
Yield (Kg/he)
July precipitation (cm)
11
10
9
8
7
22
24
26
28
30
32
Average temperature of July and August (° C)

(C2)

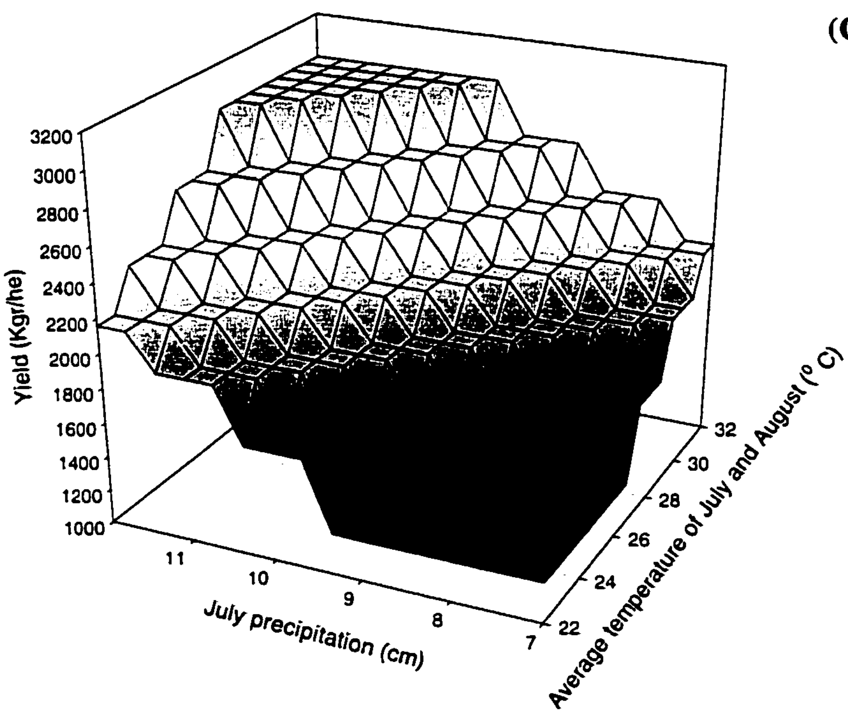
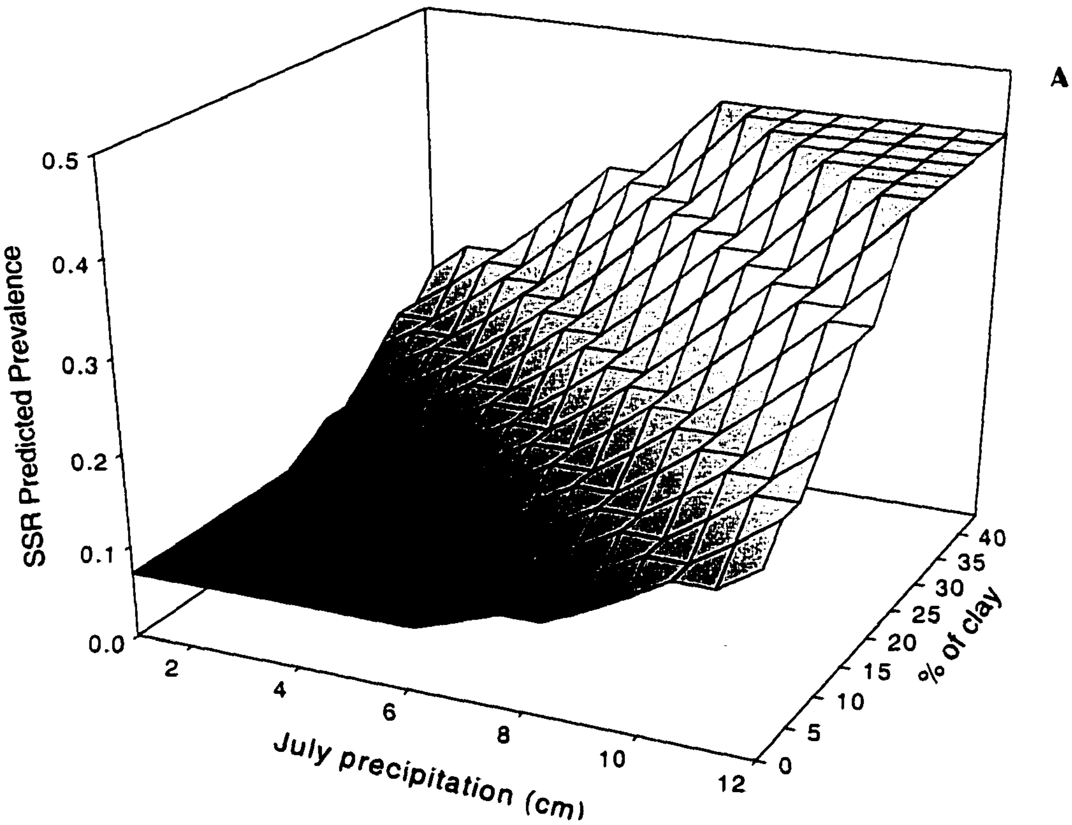

3200
3000
2800
2600
2400
2200
2000
1800
1600
1400
1200
1000
Yield (Kgr/he)
11
10
9
8
7
July precipitation (cm)
22
24
26
28
30
32
Average temperature of July and August (° C)

Fig. 6.3. **(A)** Estimated Sclerotinia stem rot (SSR) prevalence caused by *Sclerotinia sclerotiorum* in no-tilled fields with seed treatment using the estimated parameters of Tables 3-6. **(B)** Yield calculated using parameter estimates for Illinois yield quantification (Table 5) in no-tilled fields with or without seed treatment and different levels of Sclerotinia stem rot (SSR) incidence. For the calculations summer months' precipitation was considered equal to 12 cm, average temperature of July and August equal to 20 °C, and clay 5 30%. Other management practices are not considered.


A
SSR Predicted Prevalence
0.0
0.1
0.2
0.3
0.4
0.5
July precipitation (cm)
2
4
6
8
10
12
% of clay
0
5
10
15
20
25
30
35
40

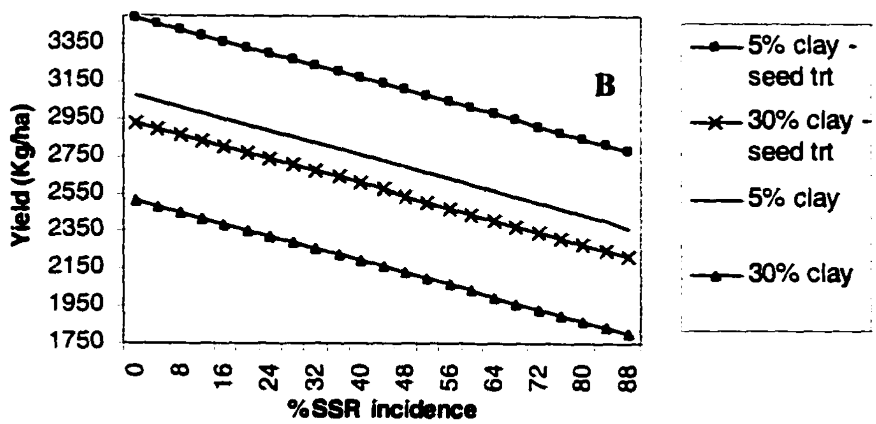

B
Yield (Kg/ha)
3350
3150
2950
2750
2550
2350
2150
1950
1750
%SSR incidence
0
8
16
24
32
40
48
56
64
72
80
88
5% clay - seed trt
30% clay - seed trt
5% clay
30% clay

Table 1. List of variables used in soybean Sclerotinia Stem Rot (caused by *Sclerotinia sclerotiorum*) prevalence model and yield quantification in the North Central Region of the United States

Variable type	Variable description	Category definitions *	Unit
Management practices			
	Seed treatment	0: no seed trt, 1: seed trt	(None)
	Manure	0: no manure, 1: manure	(None)
	Fertilizer	0: no fertilizer, 1: fertilizer application	(None)
	Herbicide application	0: no herbicide, 1: herbicide application	(None)
	No-till (1)	0: minimun or conventional till, 1: no-till	(None)
	Min-till (1)	0: odd passes, 1: even passes	(None)
	Conventional till (1)	0: more than 3, 1: one, 2: two, 3: three passes	(None)
	Tillage (2)	1: no-till, 2: min-till, 3: conventional till	(None)
Weather			
	Average air temperature of July and August		(^{0}C)
	Air temperature of September (2)		(^{0}C)
	June precipitation		(cm)
	July precipitation		(cm)
	August precipitation		(cm)
	September precipitation (2)		(cm)
Soil texture			
	% of clay		(None)
	% of silt		(None)
	% of sand		(None)
Sclerotinia Stem Rot (SSR)			
	Prevalence	0: no, 1: yes	(None)
	Incidence (2)		(None)
Other			
	Erosion	0: no, 1: yes	(None)
Yield			(Kg/ha)

* only for categorical variables.

(1) Used only in the SSR prevalence model.

(2) Used only in the yield model.

Table 2. Contingency tables analysis of association between categorical variables that represent application of management practic the fields sampled from Illinois, Iowa, Minnesota and Ohio in 1995 and 1996. The null hypothesis of independence between two categorical variables is tested with a chi-square test

	X^2	P	X^2	P	X^2	P	X^2	P	X^2	P
	Minimum tillage		No-tillage		Conventional tillage		Seed treatment		Manure	
Herbicide application (IA)	62	<0.0001	60.3	<0.0001	0.2	0.63	7.5	0.006	8.4	0.015
Herbicide application (MN)	4.85	<0.027	28.1	<0.0001	20.6	<0.0001	10.7	0.001	5.7	0.056
Fertilizer (IL)	0.57	<0.45	0.33	<0.56	0.2	0.66	0.41	0.52	26.6	<0.0001
Fertilizer (IA)	31.9	<0.0001	6.84	0.009	3.9	0.04	17.1	<0.0001	17.4	0.0002
Fertilizer (MN)	4.77	<0.029	37.7	<0.0001	34.4	<0.0001	4.8	0.027	7.7	0.021
Fertilizer (OH)	34.4	<0.0001	71	<0.0001	0.3	0.59	68.3	<0.0001	14.7	0.0006
Manure	38.4	<0.0001	28.2	<0.0001	0.4	0.82	4.1	0.12	...	
Seed treatment	4.2	<0.039	5.3	<0.022	0	0.99	...		...	

Table 3. *t*-tests of the null hypothesis of no significant difference of the values of precipitation of June (Junpr), July (Julpr), August (Augpr) and September (Seppr) between the categories of each categorical variable that represent application of a management practice in the fields sampled from Illinois, Iowa, Minnesota and Ohio in1995 and 1996

	Junpr	Julpr	Augpr	Seppr
Seed treatment	0.2	0.35	3.1*	2.85*
Manure (liquid)	2.9*	1.49	0.01	2.95*
Manure (dry)	0.46	1.06	2.05*	1.92
No-till	0.23	1.83	6.05*	2.56*
Minimum-till	2.95*	0.08	6.4*	0.38
Conventional-till	2.06*	1.83	1.7	0.54
Fertilizer (IL)[a]	0.22	0.2	2.16*	13.1*
Fertilizer (IA)[a]	0.76	0.68	0.25	42.8*
Fertilizer (MN)[a]	4.5*	1.35	0.01	3.37*
Fertilizer (OH)[a]	1.94	0.47	1.11	3.03*
Weed cultivation (IA)[a]	2*	3.3*	5*	44.4*
Weed cultivation (MN)[a]	3.14*	0.2	2.6*	10.2*

[a] IL: Illinois, IA: Iowa, MN: Minnesota, OH: Ohio.

* Significant *t*-values at 5% level of significance.

Table 4. Parameter estimates of management practices and summer weather effects on the prevalence of soybean Sclerotinia stem rot (SSR), caused by *Sclerotinia sclerotiorum*, and on soybean attainable yield in Iowa for 1995 and 1996

SSR Prevalence Model			*Yield Quantification*		
Variables	Parameter Estimates	Standard Error	Variables	Parameter Estimates	Standard Error
Seed treatment	-1.96*	0.83	Intercept	98.36*	18.18
Manure (liquid)	3.8***	2.23	SSR incidence (%)	-6.4*	1.72
(July precipitation) x (no-till)	0.19**	0.09	Average temperature of July and August	-2.7*	0.74
Min-tillage(even against odd passes)	-0.63*	0.21	June precipitation	0.5*	0.17
			July precipitation	0.81*	0.24
No-till	-2***	1.18	August precipitation	0.28*	0.09
(seed treatment) x (clay)	0.055**	0.025	September precipitation	-0.4*	0.12
			Erosion	-1.66**	0.91
			Manure (liquid)	4**	2.11
			No-till	4.56***	2.62
			Min-till	4.7**	2.23
			(Average temperature of July and August) x (SSR incidence)	0.3*	0.08
			(Average temperature of July and August) x (Herbicide application)	1.16***	0.64
			(June precipitation) x (no-till)	-0.53*	0.28
			(June precipitation) x (min-till)	-0.4**	0.19
			(July precipitation) x (clay)	-0.028*	0.007
			(July precipitation) x (seed treatment)	0.51*	0.21

Significant at: * 1%, ** 5%, and ***10% levels.

Table 5. Parameter estimates of management practices and summer weather effects on the prevalence of soybean Sclerotinia stem rot (SSR), caused by *Sclerotinia sclerotiorum*, and on soybean attainable yield in Ohio for 1995 and 1996

SSR Prevalence Model

Variables	Parameter Estimates	Standard Error
Seed treatment	-1.96*	0.83
Manure (liquid)	3.8***	2.23
(July precipitation) x (no-till)	0.19**	0.09
Min-till(even against odd passes)	-0.63*	0.21
No-till	-2***	1.18
(seed treatment) x (clay)	0.055**	0.025

Yield Quantification

Variables	Parameter Estimates	Standard Error
Intercept	31.96*	18.18
SSR incidence (%)	-6.4*	1.72
Average temperature of July and August	0.19*	1.03
July precipitation	0.81*	0.24
August precipitation	-0.19*	0.16
September precipitation	-0.4*	0.12
Erosion	-1.66**	0.91
Manure (liquid)	4**	2.11
No-till	4.56***	2.62
Min-till	4.7**	2.23
(Average temperature of July and August) x (SSR incidence)	0.3*	0.08
Herbicide application	-9.08*	3.82
Fertilizer	-9.8*	3.77
(June precipitation) x (no-till)	-0.53*	0.28
(June precipitation) x (min-till)	-0.4**	0.19
(July precipitation) x (clay)	-0.028*	0.007
(July precipitation) x (seed treatment)	0.51*	0.21
(June precipitation) x (fertilizer)	0.73**	0.32
(September precipitation) x (Herbicide application)	1.58*	0.66

Significant at: * 1%, ** 5%, and ***10% levels.

Table 6. Parameter estimates of management practices and summer weather effects on the prevalence of soybean Sclerotinia stem rot (SSR), caused by *Sclerotinia sclerotiorum*, and on soybean attainable yield in Illinois for 1995 and 1996

SSR Prevalence Model			*Yield Quantification*		
Variables	Parameter Estimates	Standard Error	Variables	Parameter Estimates	Standard Error
Seed treatment	-1.96*	0.83	Intercept	98.36*	18.18
Manure (liquid)	3.8***	2.23	SSR incidence (%)	-6.4*	1.72
(July precipitation) x (no-till)	0.19**	0.09	Average temperature of July and August	-2.7*	0.74
Min-till(even against odd passes)	-0.63*	0.21	July precipitation	0.81*	0.24
			August precipitation	0.28*	0.09
No-till	-2***	1.18	September precipitation	-0.4*	0.12
(seed treatment) x (clay)	0.055**	0.025	Erosion	-1.66***	0.91
			Manure (liquid)	4**	2.11
Fertilizer	-1.67***	1.01	No-till	4.56***	2.62
(August precipitation x (fertilizer)	0.72***	0.44	Min-till	4.7**	2.23
			(Average temperature of July and August) x (SSR incidence)	0.3*	0.08
			(June precipitation) x (no-till)	-0.53*	0.28
			(June precipitation) x (min-till)	-0.4**	0.19
			(July precipitation) x (clay)	-0.028*	0.007
			(July precipitation) x (seed treatment)	0.51*	0.21
			(SSR incidence) x (fertilizer)	-1.11*	0.41
			(September precipitation) x (Herbicide application)	0.69**	0.34

Significant at: * 1%, ** 5%, and ***10% levels.

Table 7. Parameter estimates of management practices and summer weather effects on the prevalence of soybean Sclerotinia stem rot (SSR),caused by*Sclerotinia sclerotiorum*, and on soybean attainable yield in Minnesota for 1995 and 1996

SSR Prevalence Model

Variables	Parameter Estimates	Standard Error
Seed treatment	-1.96*	0.83
Manure (liquid)	3.8***	2.23
(July precipitation) x (no-till)	0.19**	0.09
Min-till(even against odd passes)	-0.63*	0.21
No-till	-2***	1.18
(seed treatment) x (clay)	0.055**	0.025
(Fertilizer) x (June precipitation)	-0.12*	0.05
(Herbicide application) x (Aver temp of July and August)	-0.36**	0.16
Herbicide application	8.35*	3.43

Yield Quantification

Variables	Parameter Estimates	Standard Error	Variables	Parameter Estimates	Standard Error
Intercept	-13.37*	27.35	June precipitation	0.82*	0.26
SSR incidence (%)	-6.4*	1.72	Manure (liquid)	4**	2.11
Average temperature of July and August	-2.01*	1.23	No-till	4.56***	2.62
			Min-till	4.7**	2.23
(Average temperature of July and August) x (fertilizer)	2.89***	1.58	Fertilizer	-67.35**	34.38
			Herbicide application	91*	27.5
(Average temperature of July and August) x (Herbicide application)	-3.96*	1.27	Erosion	-1.66**	0.91
			July precipitation	0.81*	0.24
(Average temperature of July and August) x (SSR incidence)	0.3*	0.08	August precipit.	0.28*	0.09
			September precipit.	-0.4*	0.12
(June precipitation) x (No-till)	-0.53*	0.28			
(June precipitation) x (min-till)	-0.4**	0.19			
(July precipitation) x (clay)	-0.028*	0.007			
(July precipitation) x (seed treatment)	0.51*	0.21			
(September precipitation) x (Herbicide application)	-0.74**	0.37			

Significant at: * 1%, ** 5%, and ***10% levels.

Table 8. *Pay-off* table representing the net profit with applying or not applying no-till, minimum-till, manure, or using treated s eed in Iowa and zero, low or high Sclerotinia stem rot (SSR) incidence. Parameter estimates of Table 3 were used for the calculatic

			SSR Incidence 0%		10%		60%	
		SSR Prevalence	Yield (Kg/ha)	Profit/ha	Yield (Kg/ha)	Profit/ha	Yield (Kg/ha)	Profit/ha
Manure application in no-tilled fields	Yes	0.83	2369.66	$847.2	2211.08	$709.1	1418.18	$504.6
	No	0.24	2211.08	$796.2	2052.50	$739.1	1259.60	$453.6
Cost of manure application (/ha)	*$6.07*							
Seed treatment in no-tilled fields	Yes	0.78	2369.66	$851.7	2211.08	$794.6	1418.18	$509.1
	No	0.80	2248.35	$809.6	2089.77	$752.5	1296.87	$467.0
Cost of seed treatment (/ha)	*$1.60*							
Manure application and seed treatment in no-tilled fields	Yes	0.80	2369.66	$845.6	2211.08	788.5	1418.18	$503
	No	0.30	2089.77	$752.5	1931.19	695.4	1138.29	$410
Cost of seed treatment and manure application (/ha)	*$7.67*							
No-till		0.69	2089.77	$752.46	1931.19	$695.36	1138.29	$409.86
Min-till (even passes)		0.73	2126.24	$(771.3-2.6**n*)	1967.66	$(714.2-2.6**n*)	1174.76	$(428.7-2.6**n*)
Min-till (odd passes)		0.76	2126.24	$(771.3-2.6**n*)	1967.66	$(714.2-2.6**n*)	1174.76	$(428.7-2.6**n*)
Cost of one pass in min-till (/ha)	*$2.60*							

* *n* : number of passes in min-till.

** Precipitation for every month has been set at 6 cm, average air temperature of July and August at 20 ^{o} C, and clay at 30%.

*** Soybean price used for the profit calculations was the average price of the period 1980-2000 received by Iowa farmers in October ($ 5.71/bu).

Table 9. Relationship between summer weather, management practices and prevalence of Sclerotinia stem rot (SSR), caused by *Sclerotinia sclerotiorum*, or summer weather, management practices and attainable soybean yield in four states (Illinois, Iowa, Minnesota, and Ohio) of the North-Central Region of the United States.

	SSR prevalence model	*Yield Quantification*	
Average temperature of July and August	-- *(1)	-- (1)	Average temperature of July and August
July precipitation	+ **(1)	+ (1)	July precipitation
Seed treatment	--	+	Seed treatment
Manure (liquid)	+	+	Manure (liquid)
No-till (comparing to conventional tillage)	-- (1), (2)	-- (1), (2)	No-till (comparing to conventional tillage)
Minimum-till (comparing to conventional tillage)	+ (1), (2)	-- (1), (2)	Minimum-till (comparing to conventional tillage)
Fertilizer (MN)	--	--	Fertilizer (MN)
Herbicide (MN)	+	+	Herbicide (MN)

[*] Negative relationship, [**] Positive relationship.
(1) Relationship of weather and tillage with SSR prevalence are based on results from the present study and results from references 33 and 55.
(2) Relationship of no and minimum tillage with attainable yield can be positive when precipitation of June is low.

CHAPTER VII. GENERAL CONCLUSIONS

Our results suggest that prediction of the prevalence of Sclerotinia stem rot (SSR), caused by *Sclerotinia sclerotiorum*, in the North-Central Region of the United States is feasible. Tillage practices, air temperature, and precipitation during the months of July and August account significantly for the variation in the prevalence of SSR in four states (Illinois, Iowa, Minnesota, and Ohio) of the North Central Region of the United States. Use of these variables allows for the development of models with high explanatory power. Furthermore, investigation of the effect of production practices on SSR occurrence and soybean attainable yield demonstrated that environments that promote high attainable yield are also environments favorable to SSR development. This result is in agreement with the general notion that adoption of crop practices that are intended to increase soybean yield have contributed to SSR emergence as a serious soybean problem in the North Central Region of the United States during the last decade.

Literature on *Sclerotinia sclerotiorum* suggests that temperature and moisture are two of the major factors affecting stem rot development. Experiments, however, have been conducted only on constant temperature and moisture levels. Our experiments on the effects of fluctuations in soil temperature and soil moisture on sclerotia germination, and apothecium production indicate that fluctuations affect the subsequent carpogenic germination of sclerotia of *Sclerotinia sclerotiorum*. Whereas sclerotia germination and apothecia production occur in a wide range of soil temperature and moisture fluctuations, the proportion of sclerotia that germinate and produce apothecia is greater under low temperature fluctuations and continuous saturation than the other temperature and moisture fluctuations treatments. These results may explain the difference in numbers of apothecia and SSR incidence observed in soybean fields where different tillage and widths of row planting have been applied, since these practices affect the microclimatic conditions under the soybean canopy.

Investigation of many years of weather records in New York suggested that under N.Y. conditions precipitation rather than air temperature should be the limiting factor for white mold occurrence in beans, while in the North-Central Region of the United States a study of the factors affecting SSR occurrence suggested that air temperature rather than precipitation might be the limiting factor for white mold prevalence in soybeans. When modeling SSR prevalence, the logistic regression analysis demonstrated that the absolute value of the parameter associated to average air temperature of July and August was significantly higher than the value of the parameter associated to total precipitation during the month of July, and both parameters were significantly different than zero ($P \leq 0.05$) for soybean SSR prevalence. August precipitation was not a statistically significant explanatory variable ($P = 0.05$) for the prevalence of SSR in the North-Central Region of the United States. These results suggest that temperature rather than precipitation should be the major limiting factor for soybean SSR prevalence in this region.

When uncertainty associated with parameter estimates derived from our modeling was examined using Bayesian methodology it was found that the data set used to quantify SSR prevalence contains enough information to reliably estimate the effect of the average temperature during July and August, but not the effect of total precipitation during July and August. The largest uncertainty was associated to the estimate of the parameter representing the effect of total precipitation during the month of August. During the four years of the survey, precipitation in July and August was always higher or equal to the 30-year average for the North-Central Region of the United States. This may be an indication that the data used in the analysis do not account for extreme weather regional precipitation patterns and thus the magnitude of precipitation effect on SSR prevalence might be different than the one concluded with the 4-years data. More work is needed to validate our modeling results and improve our parameter estimates.

In the current literature, it has been suggested that farmers make decisions on pest management using not only objective information (derived from historical data, surveys or other objective means) but also subjective information (i.e. personal experience). This experience is weighted; farmers usually give more weight to recent observations and less to those that occurred some time in the past. Decision on pest management is based on site-specific information, i.e. field SSR incidence. A predictor of SSR incidence would be developed using microclimatic and management data collected from individual fields as inputs.

Given that SSR pressure is currently low in the states considered in our study (Illinois, Iowa, Minnesota, and Ohio) it is rather questionable whether farmers will be willing to spend time and effort collecting information to determine field SSR incidence. If this is the case, information on SSR regional prevalence might be useful to farmers, though less precise and informative than field SSR incidence. Farmers get information on SSR risk in their region but they still have to interpret the regional SSR risk to SSR incidence within their fields, probably using their past experience with the disease. Empirical studies are needed to determine if farmers are willing to trade-off a costly, labor demanding but site-specific prediction on SSR incidence with an inexpensive but non site-specific prediction on SSR prevalence, and shed light on the usefulness of prevalence prediction to manage Sclerotinia stem rot of soybean.